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Cryptic diversity in the North American *Dromochorus* tiger beetles (Coleoptera: Carabidae: Cicindelinae): a congruence-based method for species discovery

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A fundamental problem in biodiversity science is determining the number of species in any taxon, and there is a growing awareness that cryptic diversity contributes to this problem – even in well-studied groups. Discovering cryptic species requires several lines of evidence to elucidate congruent patterns across data-types, and distinguish unrecognized species. Tiger beetles are among the most well-studied insect groups; yet few new North American species have been described since the mid-20th century, suggesting that that the number of morphologically distinct species is reaching an asymptote. We explore the possibility that more species exist in the fauna as cryptic species, by analysing a broad geographic sample of all species in the genus *Dromochorus*. We employ a 'taxonomic congruence' approach, where we first generate species hypotheses from patterns of reciprocal monophyly across the mitochondrial and nuclear datasets, and test these hypotheses through congruence with population structure, morphological measures and ecological divergence. We find broad congruence that supports eight species of *Dromochorus*, more than doubling the known diversity. We also validate a previously ambiguous taxon, and re-describe previously named species. Lastly, we identify new diagnostic morphological characters, include an updated dichotomous key and provide updated natural history/ecological characteristics for the genus and individual species.

ADDITIONAL KEYWORDS: biodiversity – congruence method – cryptic species – Dromo tiger beetles – new species – North America – taxonomy.

INTRODUCTION

> 'It is a remarkable testament to humanity's narcissism that we know the number of books in the US Library of Congress on 1 February 2011 was 22 194 656, but cannot tell you – to within an order-of-magnitude – how many distinct species of plants and animals we share our world with.'
>
> Lord Robert May of Oxford
> doi:10.1371/journal.pbio.1001130

Discovering new biodiversity, during an unprecedented rate of global extinction, is vital for all the life sciences, and the quality of human life (e.g. Millennium Ecosystem Assessment, 2005; Díaz *et al.*, 2006; McCord, 2012; Garibaldi *et al.*, 2013). A fundamental problem to our knowledge of biodiversity is the existence of 'cryptic species'; that is, species that are distinct evolutionary units, but go undetected due to physical similarity with closely related species (e.g. Smith *et al.*, 2006; Bickford *et al.*, 2007; Burns *et al.*, 2008; Janzen *et al.*, 2017).

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Cryptic species can occur in well-studied taxa with a history of taxonomic stability (e.g. Roca *et al.*, 2015), and are expected to exist in groups with poor vagility (e.g. Bond & Stockman, 2008), where morphological characteristics are obscure or lacking (e.g. Hebert *et al.*, 2004; Gwiazdowski *et al.*, 2011), and/or when rapid and recent speciation has occurred (e.g. Landry *et al.*, 2003; Mendelson & Shaw, 2005). Discovering and describing cryptic species requires several lines of complementary evidence (e.g. morphological, genetic, ecological and geographic data) to elucidate congruent patterns across data-types that distinguish previously unrecognized species (e.g. DeSalle, Egan & Siddall, 2005; Bickford *et al.*, 2007). The challenge to discover cryptic species lies in the decisions about which types of data are most likely to uncover underlying relationships, and how to integrate them in analyses.

Species delineation has been traditionally based primarily or exclusively on morphological characters for the vast majority of eukaryotic taxa, with a smaller reliance on behavioural, ecological or other characters (Dayrat, 2005). This model is implicitly based on the idea that fixed morphological differences in two or more sets of populations are the result of the splitting of gene pools from a single ancestral taxon. This method of recognizing species as entities that are consistently distinct with respect to body structures is known as the Morphological Species Concept (e.g. Cronquist, 1978). However, in more recent decades, taxonomists have incorporated molecular data into taxonomic revisions and species descriptions, and starting in the early 2000s, there has been a trend towards a heavy reliance on purely molecular data, including the use of mitochondrial DNA (mtDNA) for 'DNA barcoding' (Hebert *et al.*, 2003). This sea change presented challenges for the taxonomic community on how to best reconcile and incorporate these multiple types of data (DeSalle *et al.*, 2005). If different sets of data are to be used, how best to integrate them? For years, cladistic systematists have argued for a more synthetic 'total evidence' approach based on the Popperian philosophy that all available data should be used when making systematic inferences (e.g. Faith & Truman, 2001; Rieppel, 2005). The approach is often to incorporate independent datasets into a single concatenated analysis (Eernisse & Kluge, 1993). Another method is to employ a 'taxonomic congruence' approach, where multiple datasets are separately analysed and taxonomic hypotheses are evaluated based on the consensus of all datasets (Kluge, 1998; Padial *et al.*, 2010). Many such studies lack an objective and repeatable method for delineation of species, though some authors have produced explicit methods for species inference using three or more types of data (e.g. Bond & Stockman, 2008; Davis *et al.*, 2016). Here we propose a novel method that incorporates five datasets, including: (1) mtDNA genealogy, (2) population-level tree based on multilocus genotyping, (3) population structure analysis based on multilocus genotyping, (4) ecological divergence metrics and (5) morphology and morphometric clustering.

Tiger beetles (Coleoptera: Carabidae: Cicindelinae) are fast-running predaceous insects distributed around the globe, and are among the most popular and relatively well-known groups of insects (Knisley & Schultz, 1997). They have been studied extensively with respect to their global and regional species diversity (Pearson & Cassola, 1992), geographic distributions (e.g. Pearson *et al.*, 1997, 2015) and ecology and natural history (e.g. Hori, 1982; Pearson, 1988; Hoback *et al.*, 2000). In North America, there are 113 species formally described that are generally recognized in the most recent treatment (Pearson *et al.*, 2015). The rate at which new species are being described has slowed greatly since the first half of the 1800s (Fig. 1), and it may be that very few morphologically distinct species remain undescribed, leaving mostly cryptic species to be discovered. To date, nearly all tiger beetle species delineations are derived from morphological characters; these include the pattern of white markings (maculations), colour, position and number of setae (chaetotaxy) and, to a

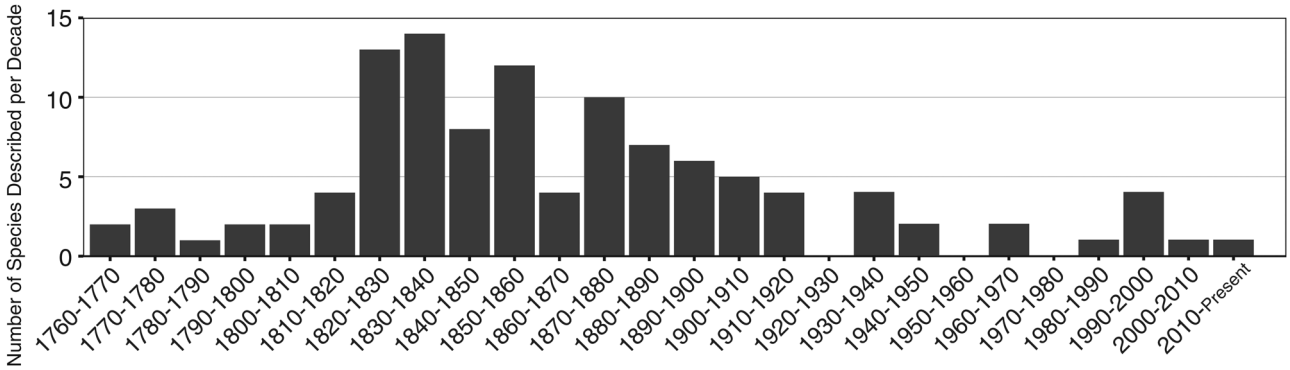

Figure 1. Number of new North American tiger beetle species described by decade, from 1766 to the present, as generally accepted in recent treatments of the North American fauna (e.g. Freitag, 1999; Pearson *et al.*, 2006; Pearson *et al.*, 2015).

lesser degree, geography. Little integrative taxonomy has been performed on the tiger beetle fauna of North America or elsewhere. Very few species descriptions/delineations incorporate non-traditional characters, although methods have been proposed to incorporate or exclusively use molecular data (e.g. Pons *et al.*, 2006). Ecological characters are almost never used (but see Duran & Roman, 2014); however, these could be considered in an integrative taxonomy framework.

The North American genus *Dromochorus* Guérin-Méneville, 1845 appears to be ideal for employing a taxonomic congruence approach, because its species are among the most taxonomically ambiguous and poorly studied groups of North American tiger beetles. Although most modern catalogues (Freitag, 1999; Erwin & Pearson, 2008; Bousquet, 2012) have recognized three or four species, these species' boundaries have never been well established (Pearson, Knisley & Kazilek, 2006), and this is likely the result of two main factors:

1. *Dromochorus* are uncommon in museum collections relative to other tiger beetles and, therefore, have been minimally available for taxonomic study. The lack of specimens probably stems from the fact that these beetles are flightless and often hide in tall grass or under trees (see Taxonomy and Species Accounts), and tend towards photophobic or crepuscular activity, making them difficult to locate. Moreover, they often occur in habitats where few or no other tiger beetles occur, and this reduces the chances that a collector will find them.
2. Taxonomy has been challenging because *Dromochorus* lack many of the morphological characters typically used to distinguish between closely related species of tiger beetles, further obscuring their intrageneric relationships. Species are black (some with blue-violet, green or pruinose sheen), lack maculations entirely and have few setae compared to other tiger beetle genera.

To explore cryptic species diversity in this group, we take a 'taxonomic congruence' approach, where multiple datasets are separately analysed and species hypotheses are evaluated based on the consensus of all datasets (Bond & Stockman, 2008; Davis *et al.*, 2016). Here we first generate species hypotheses based on patterns of reciprocal monophyly across the mitochondrial and nuclear gene datasets, and we test these hypotheses based on their congruence with population structure, conventional morphological measures, ecological divergence and geographic isolation. The main results of this integrative study are the discovery of four new *Dromochorus* species (*D. knisleyi* Duran, *et al.* **sp. nov.**, *D. welderensis* Duran, *et al.* **sp. nov.**, *D. minimus* Duran, *et al.* **sp. nov.** and *D. chaparralensis* Duran, *et al.* **sp. nov.**), the validation of one previously ambiguous taxon (*D. pruininus* Casey), and new and updated natural history/ecological characteristics for the genus and individual species. Lastly, we provide morphological descriptions of new species, re-descriptions of previously named species (*D. pilatei* Guérin-Méneville, *D. belfragei* Sallé and *D. velutinigrens* Johnson) and an updated dichotomous key to the genus.

MATERIAL AND METHODS

Specimen collection and distribution data

Historical localities for *Dromochorus* were obtained from published records and by visiting museum collections. From 2012 through 2014, we examined specimens from the following institutional and private collections (acronyms used in the text are in parentheses): American Museum of Natural History, New York, NY (AMNH); Arizona State University Frank Hasbrouck Entomology Collection, Tempe, AZ (ASUHIC); Colorado State University Insect Collection, Fort Collins, CO (CSUIC); Louisiana State University Insect Collection, Baton Rouge, LA (LSUIC); Museum of Texas Tech University Invertebrate Collection, Lubbock, TX (MTTUIC); National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (NMNH); Stephen F. Austin State University, Nacogdoches, TX (SFASU); Texas A&M University Insect Collection, College Station, TX (TAMUIC); University of Oklahoma Insect Collection, Norman, OK (UOIC); University of Texas Insect Collection, Brackenridge, TX (UTIC). Private collections used were: David P. Herrmann Collection (DPHC); Daniel P. Duran Collection (DPDC); David W. Brzoska Collection, Naples, FL (DWBC); Jeffrey A. Back Collection (JABC); John Stamatov Collection, Armonk, NY (JSC); Ronald L. Huber Collection, Bloomington, MN (RLHC); Stephen J. Roman Collection (SJRC); Walter N. Johnson Collection, Minneapolis, MN (WNJC). Additional material was field collected between 2012 and 2014 for use in both genetic analyses and morphology, with a smaller number of specimens collected in 2015 for use in morphological analyses only. All specimens and their origins are indicated in Supporting Information, Table S1. All *Dromochorus* localities that could be precisely georeferenced to within 10 km were plotted using Google Earth Pro 7.3, and converted to a .kmz file for use in ecological analyses.

Molecular sampling, mtDNA

All specimens field-collected for molecular data were preserved directly into 95–100% ethanol; when possible, museum specimens were also sampled for molecular data. DNA extractions were performed

on flight muscles removed from specimens in a non-destructive manner to preserve whole bodies for morphological observation and as voucher specimens. To do this, the head together with the pronotum were separated at the pterothorax, and flight muscles were extracted. The head and pronotum was rejoined to the rest of the body via internal water-soluble glue application (Elmer's Glue-All), not visible externally. DNA extraction was performed using Qiagen DNeasy kits per the manufacturer's protocol. A 424-bp region of the mitochondrial genome of the cytochrome *b* gene (*Cytb*) was amplified using the CB1 and CB2 primers (Crozier & Crozier, 1992). This gene was chosen based on high rate of successful amplification in the ingroup and outgroup taxa, whereas many other mtDNA primer pairs were unsuccessful. Moreover, despite its short length, it was observed that initial aligned sequences were character-rich. Primer sequences were (CB1): 5' TAT GTW YTA CCA TGA GGA CAA ATA TC 3', and (CB2): 5' ATW ACW CCT CCT AAT TTA TTA GGA AT 3'. PCR conditions were as follows: 2 min at 96 °C followed by 10 cycles of denaturation at 96 °C for 30 s, annealing at 46 °C for 30 s and extension at 72 °C for 1 min, then followed by 30 cycles of denaturation at 96 °C for 30 s, annealing at 48 °C for 30 s and extension at 72 °C for 1 min, with a final extension step at 72 °C for 5 min. PCR products were purified using either the GENECLEAN II Kit (BIO 101 Inc.) or the Millipore Multiscreen 96-well plates (Millipore, Billerica), and were sequenced using BigDye chemistry and an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Sequences were first edited manually, aligned automatically and revised by eye using SEQUENCHER v.5.2 (Gene Codes Corporation). For all individuals used in analyses, sequences for the entire 424-bp fragment were complete. Sequences were deposited in the NCBI GenBank Database, accession numbers MH410819 to MH411054.

Mitochondrial genealogy

A total of 236 specimens representing 28 geographic populations of *Dromochorus* were sampled for the mtDNA genealogy, including nine outgroup taxa in the genera *Cylindera sensu lato* and *Cicindelidia* based on a recent phylogeny of the Cicindelinae (Gough *et al.*, 2018). Bayesian phylogenetic analyses were run using BEAST v.1.7.4 (Heled & Drummond, 2010) on the XSEDE resources, via the CIPRES computer portal (Miller, Pfeiffer & Schwartz, 2010). The fragment of *Cytb* was partitioned by codon position and each partition analysed using a HKY+Γ+I substitution model (10 gamma categories), with a constant size coalescent tree prior under the assumption of a strict clock. All other priors and operators were left at default values. Samples from the posterior were taken every 1 X 10^3 steps and chains were run for 1 x 10^7 steps. Posterior distributions for each analysis were summed from two independent chains after removing the first 25% of samples as burn-in from each chain. Convergence for individual runs was visually inspected using the program TRACER v.1.5 (Rambaut & Drummond, 2007). Posterior probabilities (PP) were also evaluated in TRACER based on the effective sample size (ESS) where an ESS of >200 suggests sufficient sampling from the posterior (Ho & Lanfear, 2010). Phylogenetic trees were viewed with FIGTREE v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Multilocus marker generation and analysis

Multilocus nuclear markers were generated by genotyping a subset of specimens also analysed in the mtDNA dataset (N = 163 individuals) using a genotype-by-sequencing (GBS) approach, with reduced complexity libraries generated from a restriction enzyme procedure described in Parchman *et al.* (2012) and used in Gompert *et al.*, (2010) and Gompert *et al.* (2012). DNA sequencing of GBS libraries was performed at the University of Texas Genomic Sequencing and Analysis Facility (Austin, TX) on the Illumina HiSeq platform and yielded 194 316 108 raw paired 100-bp reads, or 38.9 total gigabases. Demultiplexing and adapter removal was performed by the *process_radtags* component of the STACKS program, v.1.41 (Catchen *et al.*, 2013). During that process, bases were discarded if they contained either uncalled bases or sliding windows averaging less than a Phred-scaled quality of 30. After filtering and demultiplexing, the average number of high-quality reads remaining for each of the 163 individuals sequenced was 718K ± 308K. The *de novo*, or non-reference-based, branch of the STACKS program pipeline (components *ustacks*, *cstacks* and *sstacks*) was used to perform local assembly and SNP identification. In *ustacks*, a maximum stack distance (parameter '-M') of 5 was used instead of the default of 2, to allow easier matching among related species; default settings were used in all other steps.

The multilocus genotype information was used to: (1) explore phylogenetic patterns among the 23 distinct sampling locations and (2) test for population genetic structure within two specific subgroups recovered in the phylogenetic analysis. To address these two goals, we generated two different working sets of loci with the *populations* component of STACKS, varying in two locus-inclusion criteria: P = the minimum number of populations in which a locus must appear; and R = the minimum fraction of individuals within a population required to possess a locus.

The first set of loci was generated with relatively relaxed criteria (P = 5, R = 0.10), in order to produce a large number of well-represented loci to inform

phylogenetic analysis. This larger set included 488K total single nucleotide polymorphisms (SNPs), of which 61 890 alleles were fixed within and variable among populations. Tree generation was based on these fixed alleles (similar to Emerson *et al.*, 2010), using components of PHYLIP v.3.696 (http://evolution.genetics.washington.edu/phylip/). A distance matrix was calculated using PHYLIP's *dnadist* program, using the F84 model with default parameters. The tree was generated by PHYLIP's *fitch* program using the Fitch–Margoliash method with global rearrangement, and visualized with FIGTREE v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

A second set of loci was generated with relatively strict criteria (P = 2 and R = 0.75) for use in population structure analysis. This second analysis required more sampling of alleles within populations to address fine-scale differences between closely related populations or species. This set included 44 645 SNPs over the 23 populations. Two subgroups on the distance-based tree invited more detailed investigation of their substructure with Bayesian clustering: a subgroup of three species (*D. belfragei*, *D. knisleyi* and *D. pruininus*) and another subgroup of four (*D. chaparralensis*, *D. minimus*, *D. velutinigrens* and *D. welderensis*). For purposes of efficient clustering, we selected a subset of 5000 SNP loci semi-randomly, by retaining approximately every 900th column from the full dataset of ~44K loci. STRUCTURE v.2.3.4 (Pritchard, Stephens & Donnelly, 2000) was then used to perform unsupervised assignment of individuals to populations within each of the two species subgroups. For each subgroup, we ran STRUCTURE with values of K from 1 to 5, at 10 replicates each, with 250 000 burn-in steps and 250 000 calculation steps per replicate, using default parameters, including the admixture model. Optimal K values were determined using STRUCTURE HARVESTER (Earl & vonHoldt, 2012) (taylor0.biology.ucla.edu/structureHarvester/).

Specimen measurements and morphometric analyses

Putative species identified from mtDNA, GBS and geography were evaluated to identify any fixed morphological differences that may be diagnostic (synapomorphies). We also measured body structures for use in subsequent morphometric analyses to discern whether putative species are significantly different in size or shape. All body measurements follow the format of Duran and Roman (2014) and are defined as follows: the total body length (TL) excludes the labrum and is measured as the distance from the anterior margin of the clypeus to the elytral apex; pronotum width (PW) is measured to include the lateral margins of the proepisterna; head width (HW) is measured as the distance between the outer margins of the eyes. Measurements were performed on recent field-collected specimens (from 2012 to 2015), or undamaged museum specimens. Many older museum specimens were unsuitable for analysis due to wear, and missing or broken structures.

PCA analysis of adult morphology

We performed a series of principal component analyses (PCA) on the set of four linear measurements and two aspect ratios collected for each of 411 individuals, using the R (v.1.3.4) *prcomp* function with log transformation, centring and scaling to generate the components and their loadings. Those measurements were: overall body length, elytra length and elytra/body length ratio; and pronotum length, pronotum width and pronotum length/width ratio. We focused on two targeted comparisons from the multilocus population phylogeny: (1) among the *D. minimus–D. velutinigrens–D. welderensis–D. chaparralensis* clade; and (2) among the *D. knisleyi–D. belfragei–D. pruininus* clade. To compare species for different average principal components of morphology, we used a nested ANOVA with *species* as a main factor and *sex within species* as a nested factor, which allowed the comparison of species while controlling for sexual dimorphism nested within species. Following ANOVA, we performed post hoc comparisons of mean PCA loading scores using a Tukey's HSD ($\alpha = 0.05$). Mean PCA loading scores ± standard errors (SE) are reported in the results.

Ecological analyses

Our observations between 2012 and 2015 suggested that putative species were specialized with respect to soil type, hydrology and plant cover. To evaluate ecological niche and ecological divergence between species, we assessed each *Dromochorus* species occurrence in Environmental Protection Agency (EPA) Level III ecoregions (Omernik & Griffith, 2014). Each ecoregion is a geographically defined area with characteristic abiotic (e.g. geologic, climate, soil type and hydrology) and biotic (primarily plant community) attributes. All geo-referenced *Dromochorus* localities were imported to the ARCGIS 10.2 software as a layer, along with shape files of EPA Level III ecoregions, and each point locality was buffered by a radius of 10 km. Radius buffer distance was estimated as a maximum dispersal distance to elucidate species distribution and potential ecological overlap, as well as to account for potential imprecision in locality data. Individual species sampling locations that overlapped in areal coverage were merged and all buffered area overlaid with EPA Level III ecoregion map layers. Per cent composition of ecoregion was calculated for each species.

Using the per cent membership in each putative *Dromochorus* species in each ecoregion (Table 1), we generated a measure of dissimilarity by calculating a distance matrix using Euclidean (standard distance between two vectors) and Manhattan (absolute distance between two vectors) distances (measured in km). To test for significance, we permuted the data in per cent membership matrix 100 times using the program PERMUTE and calculated dissimilarity with the program DISTANCE in R (v.3.4.1).

RESULTS

MtDNA genealogy

The genus *Dromochorus* was recovered as monophyletic with strong support (1.0 PP), consisting of several reciprocally monophyletic clades (Fig. 2) that are each distributed in specific geographic areas (Fig. 3). The three historically defined and generally recognized nominal species, *D. pilatei*, *D. velutinigrens* and *D. belfragei*, were each recovered as monophyletic on the mtDNA tree (1.0, 0.96 and 1.0 PP, respectively). In addition, there were geographically structured, strongly supported subclades within each of the nominal taxa *D. velutinigrens* and *D. belfragei*. These observed subclades were considered potential cryptic species and were subsequently evaluated for distinctiveness based on multilocus DNA analyses, morphology and ecology. Within the '*velutinigrens* group' there were three potential cryptic species based on reciprocal monophyly and observed allopatry of these clades (Fig. 2A). In the '*belfragei* group' there were four subclades that were identified as potentially distinct species (Fig. 2B). Two of these clades corresponded to *D. belfragei sensu stricto,* and the previously named but historically contentious taxon, *D. pruininus*; two other possible cryptic species were identified for follow-up congruence comparison to other datasets. Subsequent data would find broad congruence between putative cryptic species and the mtDNA clades, and notable polyphyly only occurred where populations of these taxa came in close geographic contact in Bexar County, Texas (vicinity of San Antonio, TX) (Fig. 2B).

Multilocus results

The PHYLIP-generated population-level tree based on 488K SNPs from our GBS approach (Fig. 4) recovered clades that were generally consistent with the mtDNA genealogy. Except for *D. belfragei/knisleyi*, all other putative species were monophyletic (congruent with the mtDNA results), although due to rarity and small sample sizes, four taxa (*D. minimus*, *D. welderensis*, *D. chaparralensis* and *D. velutinigrens*) were each represented from a single population in the multilocus analysis. As in the mtDNA genealogy, the only non-monophyly occurred in Bexar County, TX, where populations of multiple species were sympatric/parapatric. In this case, one population of *D. belfragei* was recovered in a clade of *D. knisleyi*. The only topological discordance between the population-level tree and the mtDNA tree was the placement of *D. minimus*, where all specimens appeared in the '*velutinigrens* group' in the population-level tree, and in the '*belfragei* group' in the mtDNA tree.

Table 1. *Dromochorus* species occurrence in EPA Level III ecoregions (Omernik & Griffith 2014). Values indicate the percentage of each species' presence in each of 15 ecoregions

Ecoregions (EPA Level III)	*D. weld*	*D. velu*	*D. prui*	*D. belf*	*D. chap*	*D. knis*	*D. mini*	*D. pila*
East Central Texas Plains	14.5	*0*	5.3	12.1	*0*	*0*	9.0	*0*
Western Gulf Coastal Plain	85.5	54.6	*0*	*0*	*0*	*0*	*0*	71.4
Southern Texas Plains	*0*	45.4	*0*	*0*	100	*0*	47.0	*0*
Central Great Plains	*0*	*0*	14.3	23.1	*0*	*0*	*0*	*0*
Flint Hills	*0*	*0*	20.4	*0*	*0*	*0*	*0*	*0*
Cross Timbers	*0*	*0*	6.3	27.2	*0*	*0*	*0*	*0*
Texas Blackland Prairie	*0*	*0*	26.5	18.8	*0*	6.0	44.0	*0*
South Central Plains	*0*	*0*	3.3	6.1	*0*	*0*	*0*	20.9
Central Irregular Plains	*0*	*0*	22.7	*0*	*0*	*0*	*0*	*0*
Western Corn Belt Plains	*0*	*0*	1.3	*0*	*0*	*0*	*0*	*0*
High Plains	*0*	*0*	*0*	3.9	*0*	*0*	*0*	*0*
Southwestern Tablelands	*0*	*0*	*0*	3.1	*0*	*0*	*0*	*0*
Edwards Plateau	*0*	*0*	*0*	5.8	*0*	94.0	*0*	*0*
Mississippi Alluvial Plain	*0*	*0*	*0*	*0*	*0*	*0*	*0*	3.9
Southern Coastal Plain	*0*	*0*	*0*	*0*	*0*	*0*	*0*	3.8

Abbreviations are as follows: *D. weld* = *D. welderensis*, *D. velu* = *D. velutinigrens*, *D. prui* = *D. pruininus*, *D. belf* = *D. belfragei*, *D. chap* = *D. chaparralensis*, *D. knis* = *D. knisleyi*, *D. mini* = *D. minimus*, *D. pila* = *D. pilatei*

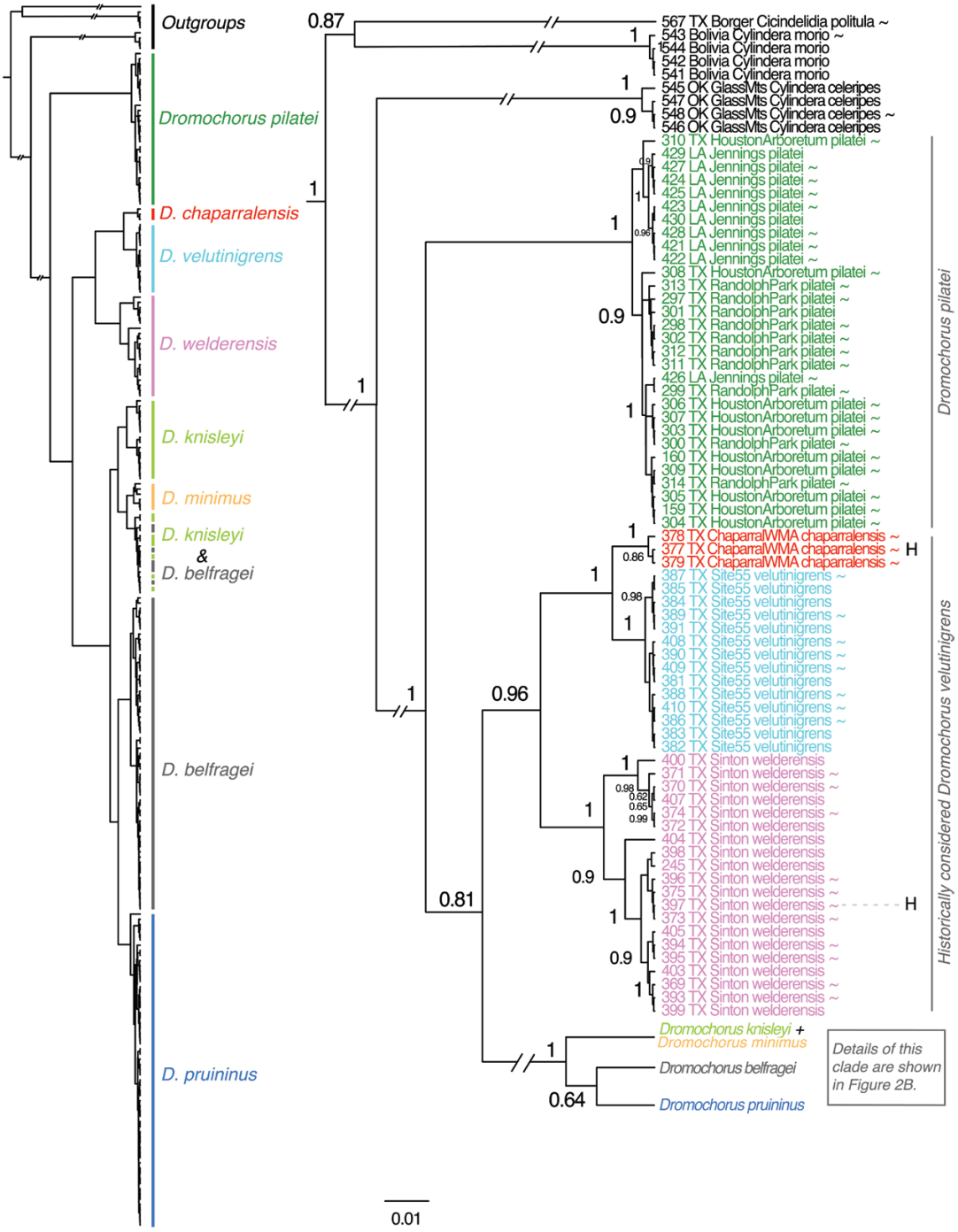

Figure 2A. Mitochondrial DNA (mtDNA) cytochrome oxidase *b* (*Cytb*) Bayesian genealogy of all members of the genus *Dromochorus*. Outgroups include North American representatives of *Cylindera* and *Cicindelidia*. On the left is a reduced version of the full tree indicating clades shown, in detail, in each figure. Colours are arbitrarily chosen to show distinctions among the taxa, and directly correspond to populations shown in Figure 3. Only posterior support values above 60 are displayed, and the scale bar at bottom shows a branch length representing nucleotide substitutions per site. Taxon names include the unique specimen number (see also Supporting Information, Table S1), along with the state and collection locality; genomic material from specimens marked with a ~ were also used in the multilocus analysis (Figure 4), and holotypes of species described in this work are marked with an 'H'. The longitudinal bars on the right identify specimens and clades historically considered to be a single species.

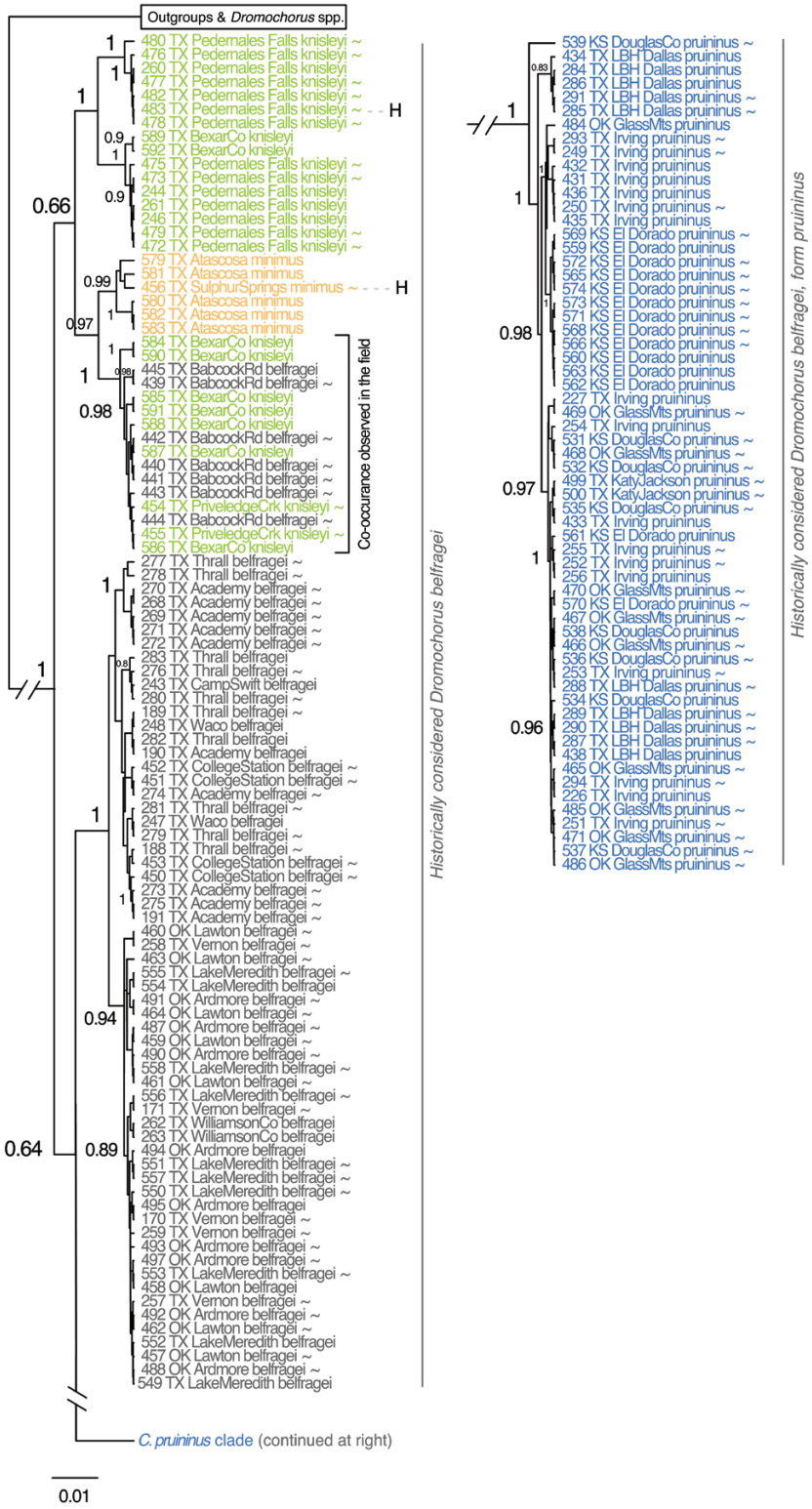

Figure 2B. This section of the mitochondrial genealogy focuses on clades historically considered to be part of a single species: *Dromochorus belfragei*. Notable is the mixed clade of specimens of *D. knisleyi* and *D. belfragei* collected from the only area where both species are known to occur.

Based on the results of the population-level tree, two sets of STRUCTURE analyses were conducted, as described in the multilocus results' section, comparing the putatively new and previously described species. The first set compared *D. chaparralensis*, *D. minimus*, *D. velutinigrens* and *D. welderensis* (Fig. 5). At K = 2, a clear separation was observed between *D. velutinigrens* + *D. welderensis* and *D. chaparralensis* + *D. minimus*; at K = 3, *D. welderensis* and *D. velutinigrens* separated, and at K = 4, *D. chaparralensis* and *D. minimus* separated. K = 5 did not yield any further significant changes to the overall population structure. The method of Evanno *et al.* (2005) based on the rate of change in the log probability of data between successive K values (delta-K) was assessed to determine the number of clusters (species) that best fit the data. This method indicated that K = 4 was best supported. A second set of STRUCTURE analyses was run comparing *D. belfragei*, *D. pruininus* and *D. knisleyi* (Fig. 5). At K = 2, individuals belonging to *D. pruininus* were largely separated from *D. belfragei* + *D. knisleyi*; at K = 3, there was a separation between all *D. knisleyi* individuals and all *D. belfragei* populations,

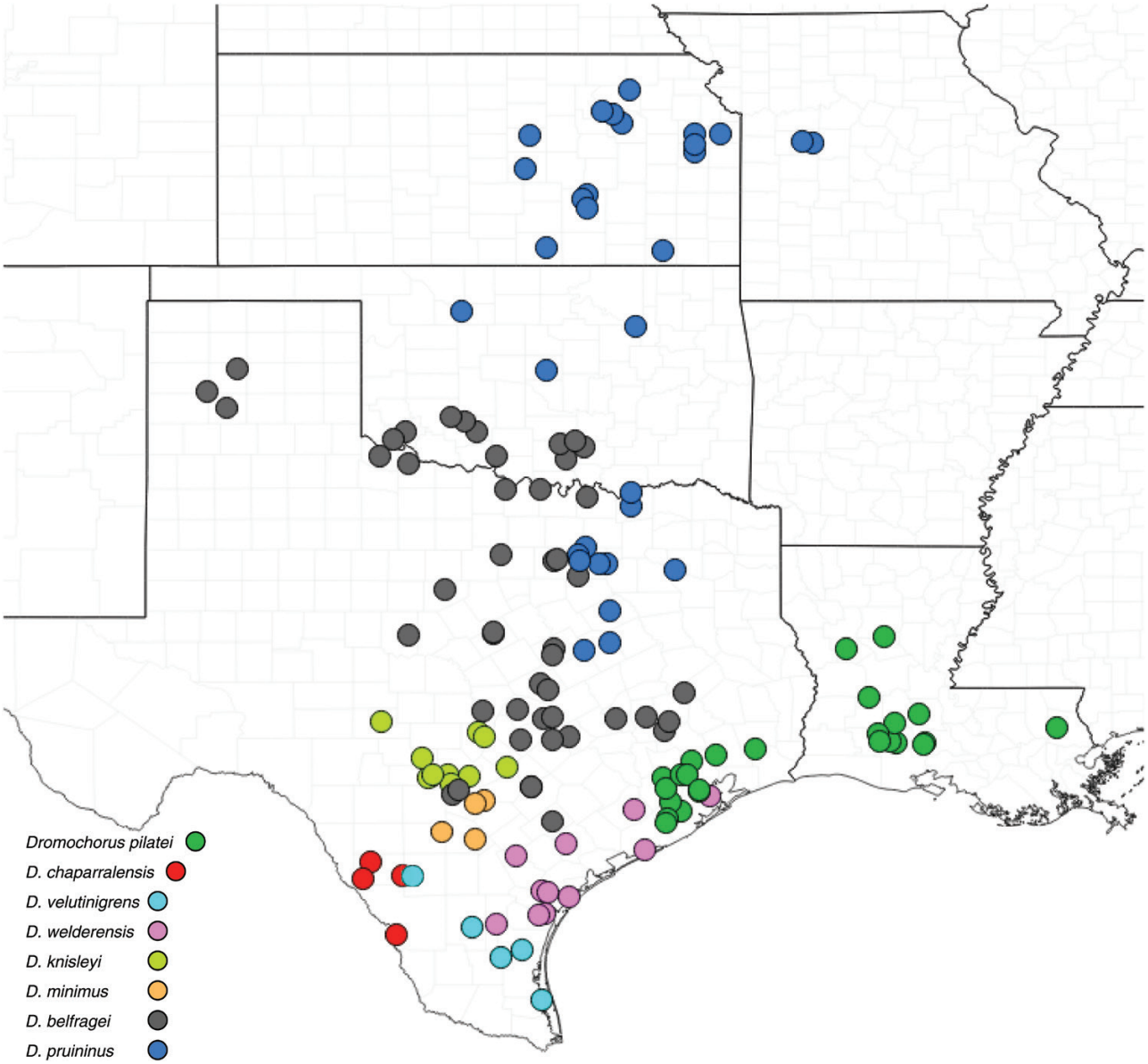

Figure 3. Distribution of sampled *Dromochorus* populations. Colours correspond to those used in Figure 1. Green = *D. pilatei*, pink = ***D. welderensis* sp. nov.**, sky blue = *D. velutinigrens*, red = ***D. chaparralensis* sp. nov.**, blue = *D. pruininus*, lime green = ***D. knisleyi* sp. nov.**, orange = ***D. minimus* sp. nov.**, black = *D. belfragei*.

except for the population San Antonio, Old Babcock Rd (Fig. 2B) from Bexar County, TX. K = 4 and K = 5 did not appreciably change the population structure in any biologically or taxonomically relevant way; however, delta-K plots indicated that K = 4 was best supported.

PCA ANALYSIS OF ADULT MORPHOLOGY

Given the natural covariation in body measurements (Table 2), PCA on the morphological data found that the first three principal components (overall body size, shape of the pronotum and elytral length to body length ratio) explained 92–95% of the total variation in the dataset. Thus, we compared these first three components in targeted contrasts of two natural groups that emerged from the genetic data. This allowed us to address hypotheses for these putative cryptic species. We found that principal component 1 scaled with overall body size, principal component 2 scaled with pronotum shape (which ranges from long and thin to short

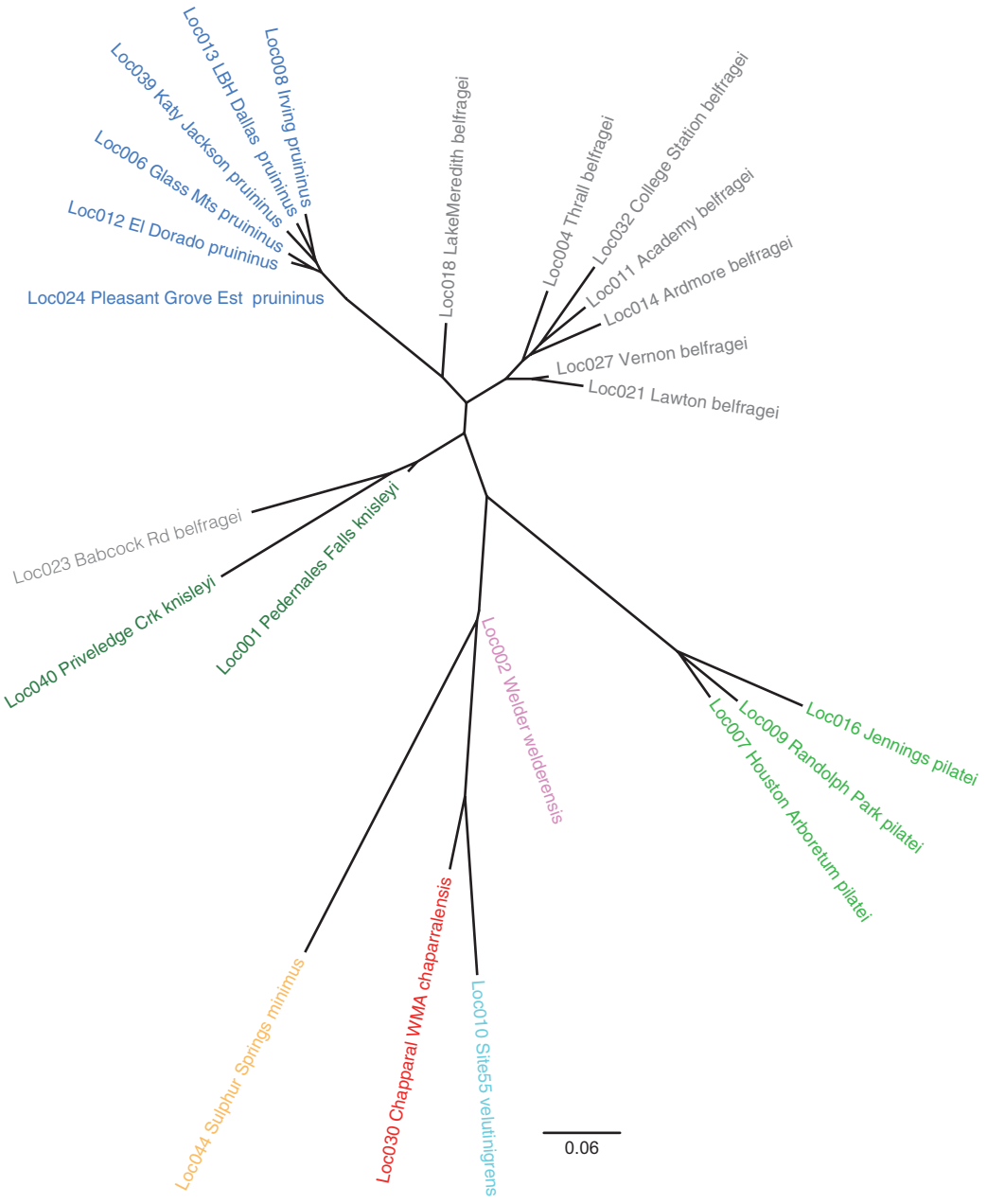

Figure 4. Unrooted population-level tree based on 61 890 GBS loci, constructed using the Fitch–Margoliash method in PHYLIP. Each branch of the tree represents a single geographic locality. Colours are as in Figures 2, 3.

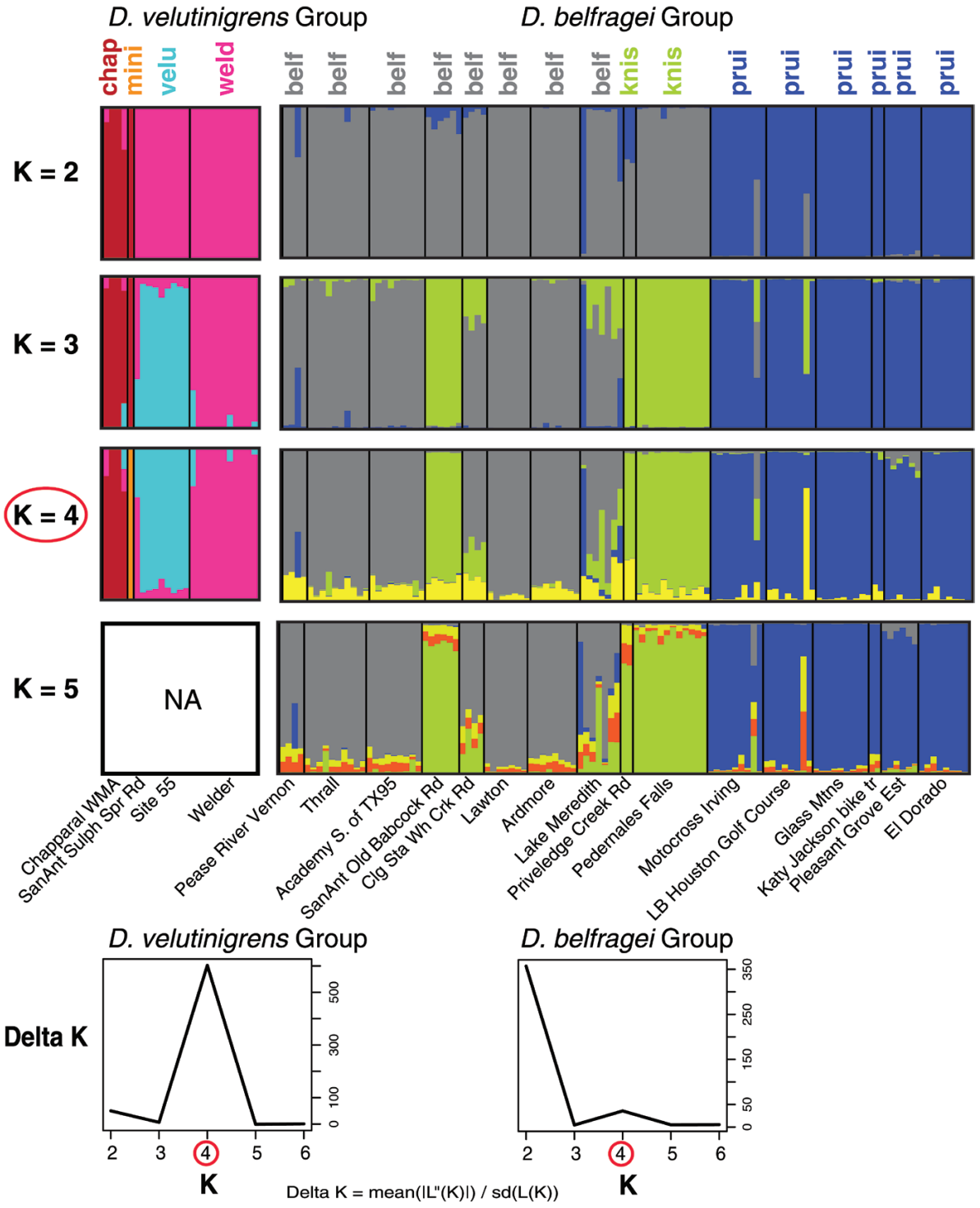

Figure 5. STRUCTURE plots based on dataset of 5000 random GBS loci. Each locus was present in at least two populations and present at greater than 75% of individuals in those populations. K-values determined by STRUCTURE *Harvester* (Earl & vonHoldt, 2012) are shown at left of the population assignments for each value, and as delta-K estimations across all values. The collection localities that correspond to the columnar groups of specimens determined by the population assignments appear at bottom with the end of the phrase adjacent to its columnar group in all assignments. Left panel, K = 2–4 for '*velutinigrens* group'. Delta-K plot indicated that K = 4 is the best supported explanation for the data. Right panel, K = 2–5 for '*belfragei* group'. Delta-K plot indicated that K = 4 is the best-supported explanation for the data.

and wide) and principal component 3 scaled with the ratio of elytra length to body length. Details for specific comparisons in both natural groups are below (Fig. 6).

PCA ON THE *D. MINIMUS–D. VELUTINIGRENS–D. WELDERENSIS–D. CHAPARRALENSIS* CLADE

Among the *minimus–velutinigrens–welderensis–chaparralensis* clade, we compared the average loading score from the PCA using ANOVA coupled with a Tukey's HSD post hoc analysis to determine which species differed ($\alpha = 0.05$; Fig. 6) and found PC1, PC2 and PC3 exhibited significant differences among species (PC1: $F_{3,135} = 19.40$, $P < 0.0001$; PC2: $F_{3,135} = 4.63$, $P = 0.0041$; PC3: $F_{3,135} = 9.72$, $P < 0.0001$).

PC1 was generally associated with body size, and the smallest species, *D. minimus* (PC1 mean ± SE: –1.01 ± 0.20), differed significantly from the two largest species, *D. chaparralensis* (PC1 mean ± SE: 1.41 ± 0.38) and *D. welderensis* (PC1 mean ± SE: 0.78 ± 0.19), which were not significantly different from each other. *Dromochorus velutinigrens* (PC1 mean ± SE: –0.24 ± 0.19) was intermediate and significantly different from both the smaller *D. minimus* and the larger pair, *D. chaparralensis* and *D. welderensis*.

For PC2, which captured pronotum shape ranging from long and thin to short and wide, we found that *D. welderensis* (PC2 mean ± SE: 0.64 ± 0.14) and *D. velutinigrens* (PC2 mean ± SE: 0.57 ± 0.14) were not different from each other, but were significantly different from *D. minimus* (PC2 mean ± SE: 0.01 ± 0.15), with *D. chaparralensis* (PC2 mean ± SE: -0.01 ± 0.29) being more variable and not different from any other species.

For PC3, which captured the relative length of the elytra to the length of the body, we found that *D. velutinigrens* (PC3 mean ± SE: 0.12 ± 0.14) and *D. minimus* (PC3 mean ± SE: –0.38 ± 0.15) were not different from each other, but exhibited shorter elytra relative to their body length than *D. welderensis* (PC3 mean ± SE: –0.93 ± 0.14). Again, *D. chaparralensis* (PC3 mean ± SE: –0.68 ± 0.28) was intermediate, but not significantly different from any other species.

PCA ON *D. KNISLEYI–D. BELFRAGEI–D. PRUININUS* CLADE

Among the *D. knisleyi–D. belfragei–D. pruininus* clade, we found that PC1 and PC2 exhibited significant differences among species (PC1: $F_{2,112} = 31.49$, $P < 0.0001$; PC2: $F_{2,112} = 8.45$, $P = 0.0004$), whereas PC3 was not different among species ($F_{2,112} = 1.16$, $P = 0.3267$). We used a Tukey's HSD test to determine which species differed ($\alpha = 0.05$; Fig. 6).

For PC1, which was generally associated with body size, all three species were significantly different from each other. *Dromochorus belfragei* (PC1 mean ± SE: 2.09 ± 0.19) was the largest species, *D. knisleyi* (PC1 mean ± SE: –0.17 ± 0.22) was the smallest species and *D. pruininus* (PC1 mean ± SE: 1.47 ± 0.16) was intermediate between the two.

For PC2, which captured pronotum shape ranging from long and thin to short and wide, we found that *D. belfragei* (PC2 mean ± SE: 0.64 ± 0.17) and *D. pruininus* (PC2 mean ± SE: 0.71 ± 0.15) were not different from each other, but had significantly longer and thinner pronotum shape than *D. knisleyi* (PC2 mean ± SE: –0.37 ± 0.21).

Table 2. Mean ± SE for morphological measurements of body size and dimensions

Species	Sex	N	Body Length (BL)	Elytra Length (EL)	EL:BL Ratio	Pronotum Length (PL)	Pronotum Width (PW)	PL:PW Ratio
D. belfragei	F	15	14.51 ± 0.41	8.85 ± 0.01	0.61 ± 0.01	3.08 ± 0.04	3.39 ± 0.03	1.10 ± 0.01
	M	28	13.16 ± 0.11	8.17 ± 0.08	0.62 ± 0.01	2.83 ± 0.03	3.08 ± 0.04	1.09 ± 0.01
***D. chaparralensis* sp. nov.**	F	6	13.68 ± 0.26	8.20 ± 0.21	0.60 ± 0.01	3.08 ± 0.10	3.35 ± 0.08	1.09 ± 0.02
	M	5	12.82 ± 0.23	7.65 ± 0.16	0.60 ± 0.01	3.00 ± 0.15	3.10 ± 0.07	1.04 ± 0.02
***D. knisleyi* sp. nov.**	F	11	13.03 ± 0.21	7.88 ± 0.14	0.60 ± 0.01	2.93 ± 0.06	3.02 ± 0.07	1.03 ± 0.01
	M	14	12.18 ± 0.17	7.49 ± 0.13	0.62 ± 0.01	2.74 ± 0.05	2.81 ± 0.04	1.03 ± 0.02
***D. minimus* sp. nov.**	F	16	12.67 ± 0.19	7.57 ± 0.13	0.60 ± 0.01	2.81 ± 0.05	2.99 ± 0.04	1.07 ± 0.01
	M	27	11.68 ± 0.11	7.09 ± 0.07	0.61 ± 0.01	2.66 ± 0.03	2.73 ± 0.03	1.03 ± 0.01
D. pilatei	F	38	13.32 ± 0.09	8.15 ± 0.06	0.61 ± 0.01	2.75 ± 0.03	2.92 ± 0.02	1.06 ± 0.01
	M	83	12.37 ± 0.06	7.62 ± 0.04	0.62 ± 0.01	2.60 ± 0.02	2.70 ± 0.01	1.04 ± 0.01
D. pruininus	F	18	13.97 ± 0.12	8.50 ± 0.10	0.61 ± 0.01	2.95 ± 0.04	3.25 ± 0.03	1.10 ± 0.02
	M	32	13.14 ± 0.09	8.04 ± 0.06	0.61 ± 0.02	2.70 ± 0.03	3.04 ± 0.02	1.09 ± 0.01
D. velutinigrens	F	21	13.34 ± 0.16	8.19 ± 0.10	0.61 ± 0.02	2.79 ± 0.03	2.85 ± 0.03	1.02 ± 0.01
	M	24	12.73 ± 0.12	7.71 ± 0.08	0.61 ± 0.01	2.67 ± 0.04	2.66 ± 0.04	0.99 ± 0.01
***D. welderensis* sp. nov.**	F	20	13.70 ± 0.12	8.14 ± 0.08	0.59 ± 0.01	3.14 ± 0.03	3.21 ± 0.03	1.02 ± 0.01
	M	24	12.61 ± 0.13	7.42 ± 0.09	0.59 ± 0.01	2.86 ± 0.04	2.91 ± 0.03	1.02 ± 0.01

Ecological analyses

Dromochorus inhabit 15 separate EPA Level III ecoregions (as defined in Omernik & Griffith, 2014) (Table 1) and, except for *D. chaparralensis*, each species was found to occur in two or more. Three species were found to occur nearly entirely in one ecoregion (>85%): *D. pilatei*, *D. welderensis* and *D. knisleyi*. Two species, *D. belfragei* and *D. pruininus*, inhabit the greatest number of ecoregions, each being found in eight. More species were found in the East Central Texas Plains and Texas Blackland Prairie than any other ecoregion (four species each).

Two calculations of distance were performed: Euclidean and Manhattan (Table 3). Each estimate of ecological dissimilarity generated similar results, which were highly correlated (Mantel test: $r_m = 0.8545$,

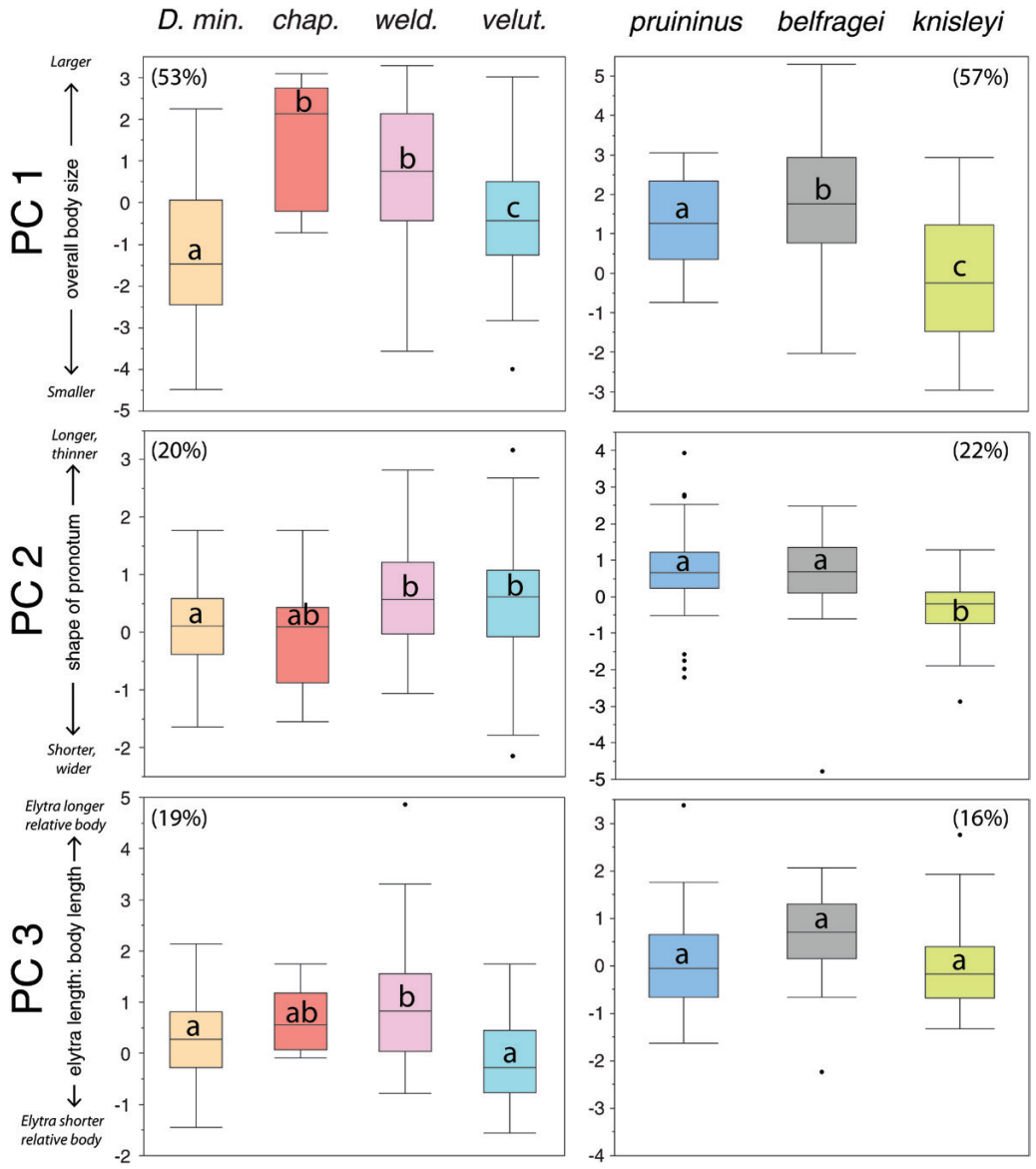

Figure 6. Boxplots, by species, from morphometric data used in principal component analyses (PCA) for each of 411 individuals. PCA indicated three measurements: PC1, overall body length; PC2, shape of the pronotum; and PC3, elytral length to body length ratio, to explain 92–95% of the total variation in the dataset. Shown are targeted comparisons among two main phylogenetic groups: 1, the *D. minimus–D. velutinigrens–D. welderensis–chaparralensis* group; and 2, the *D. knisleyi–D. belfragei–D. pruininus* clade. Species labelled with the same letters did not differ significantly from each other, species labelled with 'ab' did not differ between other species which were significantly different. Percentages are relative contributions per analyses, and group; outliers are shown as black dots.

Table 3. Distance matrix for ecological differentiation. Euclidean distances are on top; Manhattan distances on bottom. All distance measurements are based on values obtained from EPA Level III ecoregion data, also reported in Table 1.

	D. weld	*D. velu*	*D. prui*	*D. belf*	*D. chap*	*D. knis*	*D. mini*	*D. pila*
D. weld	0	56.8	96.4	95.1	132.4	128.0	107.2	29.6
D. velu	90.8	0	83.4	83.1	77.2	118.0	70.7	53.0
D. prui	189.5	200.1	0	40.2	109.2	102.3	61.0	85.7
D. belf	175.9	200.1	104.2	0	108.9	97.1	65.0	84.7
D. chap	200.0	109.2	200.1	200.1	0	137.4	69.5	124.8
D. knis	200.0	200.0	188.1	176.5	200.0	0	112.1	120.2
D. mini	182.0	109.2	136.5	144.5	106.0	188.0	0	98.9
D. pila	57.2	90.8	193.5	187.9	200.0	200.0	200.0	0

Abbreviations are as follows: *D. weld* = *D. welderensis*, *D. velu* = *D. velutinigrens*, *D. prui* = *D. pruininus*, *D. belf* = *D. belfragei*, *D. chap* = *D. chaparralensis*, *D. knis* = *D. knisleyi*, *D. mini* = *D. minimus*, *D. pila* = *D. pilatei*.

N = 28 pairwise comparisons, $P < 0.001$), and hereafter values given will refer to Euclidean distance. We tested for significant dissimilarity with a permutation test, where we shuffled the values in the ecoregion dataset 100 times and recalculated the distance matrix. Mean distance in the shuffled matrices was 8.99 ± 5.32 SE (95% CI = –1.92 to 19.9). All distances outside of the 95% CI range were considered significantly different, which included all actual values in the matrix (see Table 3). Species varied in the magnitude of their ecological differentiation from other geographically nearby/parapatric species. For example, *D. belfragei* exhibited less differentiation from *Dromochorus. pruininus* (Euclidean distance = 40.2) compared to its differentiation from *D. knisleyi* (97.1). *Dromochorus chaparralensis* exhibited lowest ecological differentiation from *D. minimus* (69.5) and *D. velutinigrens* (77.2), but high differentiation from *D. welderensis* (132.4), the only species from which it is morphologically indistinct.

TAXONOMY AND SPECIES ACCOUNTS

Tribe Cicindelini Latreille, 1802

Genus *Dromochorus* Guérin-Méneville, 1845

Type species

Dromochorus pilatei Guérin-Méneville, 1845. By monotypy.

Taxonomic history

Dromochorus was described by Guérin-Méneville (1845) as a new genus, believed to be most closely related to '*Apteroessa* and especially *Dromica*'. This perceived relatedness was due to the remarkable similarity in *gestalt*, although several morphological differences were identified, especially with respect to the mouthparts, such as the maxillary and labial palpi, and labrum. Subsequent authors have not shared his view about the systematic placement of the genus within the larger tiger beetle clade. In his revision of the Cicindelinae, Horn (1908) placed *Dromochorus* in the subtribe Cicindelina, and *Dromica* in a separate subtribe, Prothymina (Horn 1910). More recent molecular phylogenies have supported Horn's (1908) treatment, finding *Dromochorus* to be nested within a clade of other Nearctic Cicindelina (Vogler & Welsh, 1997; Barraclough & Vogler, 2002; Pons *et al.*, 2004; Vogler *et al.*, 2005), and *Dromica* to be more related to *Prothyma* (Galián, Hogan & Vogler, 2002). Any similarities between *Dromochorus* and *Dromica* must, therefore, be the result of convergent evolution owing to their similar habitats and microhabitats.

The *Dromochorus* species have sometimes been treated as a distinct genus (Guérin-Méneville, 1845; Sallé, 1877; Fleutiaux, 1892; Casey, 1897; Rivalier, 1954, 1963, 1971; Johnson, 1991; Wiesner, 1992; Erwin & Pearson, 2008; Bousquet, 2012; Pearson *et al.*, 2015); or viewed to be a synonym of *Cicindela* (e.g. LeConte, 1875; Leng, 1902; Harris, 1911; Horn, 1915; Harris & Leng, 1916; Cazier, 1954; Arnett, 1963; Willis, 1968; Bousquet & Larochelle, 1993; Arnett & Thomas, 2000), with some North American workers recognizing the taxon as a subgenus (Boyd, 1982; Freitag, 1999). Despite the variety of treatments, justifications for different taxonomic ranks were almost never given.

Recent phylogenies have given support to many of the named clades within the *Cicindela sensu lato* (Pons *et al.*, 2004; Vogler *et al.*, 2005; Gough *et al.*, in review), and these generally correspond to groups recognized in Rivalier's revision (1954, 1963, 1971), including the *Dromochorus*. Based on the aforementioned research, and our own mtDNA tree (Fig. 2), *Dromochorus* was recovered as a well-supported monophyletic lineage, and sister to another clade of Nearctic tiger beetles. As such, we recognize the group as a distinct genus. Although *Dromochorus* may appear nested within *Cylindera* in our

tree, this latter group is polyphyletic (Gough *et al.*, 2018), as previously believed by many workers, and the sister clade to *Dromochorus* is being named as a new genus.

Diagnosis

Dromochorus are separable from all other North American tiger beetle genera by the following combination of characters, present in the adult stage. Beetles are flightless and lack flight wings, but the elytra are not fused. The elytra are oval shaped, and completely lacking pale maculations. The dorsum is dark, usually black or brown, but may also have a frosted blue, violet, grey or green sheen. The legs and tarsi are clothed in decumbent setae.

In subsequent species descriptions, the most salient or diagnostic characters are indicated in bold. Rarely are any of these characters diagnostic by themselves, but the combination of these characters may be.

Distribution

Dromochorus are geographically restricted to the south-central United States and adjacent Mexico (Fig. 3). Texas is the centre of diversity, and all eight species are found there, at least in part of their ranges. Records from Mexico are few and imprecise. Historically, *D. belfragei* was the only species recorded from Mexico, although it would appear as if these populations belong to *D. chaparralensis* **sp. nov.**, described in this treatment. In general, *Dromochorus* are found at low elevations; apparently, they do not occur in montane environments, such as the Ouachita Mountains of Arkansas and Oklahoma, which border a section of the eastern range of *D. pruininus*. Most *Dromochorus* are found below 500 m, with only the westernmost populations of *D. belfragei* occurring at elevations up to 1000 m.

Ecology / natural history

Dromochorus appear to have a 2-year life cycle, based on observations of *D. pruininus* (Herrmann & Duran, unpublished). Adult beetles are terrestrial predators of small invertebrates, and are generally believed to be crepuscular or active in the late afternoon, but we have found them at all hours of the day in more shaded microhabitats or when significant cloud cover is present. *Dromochorus* are extremely fast runners and may evade capture by darting into dense grasses, or in some species, hiding in cracks in the earth, especially *D. belfragei* and *D. pruininus*. Larvae are poorly known, and were only recently described (Spomer, Nabity & Brust, 2008).

There are remarkably few specimens of these beetles in major museum collections, even though several species occur near major cities and universities in Texas and Oklahoma. Many species records are based on one or a few specimens, and as such, *Dromochorus* were reported to be rare, or at low densities. This is likely a consequence of their atypical natural history compared to other diurnal North American tiger beetles, and the fact that their habitats are not as commonly visited by collectors. We have found that *Dromochorus* can be remarkably abundant in the appropriate habitat during the ideal time of year, rivalling or exceeding densities observed in some common riparian tiger beetle species. These observations are unlikely to represent unusual population explosions, as we have witnessed similarly large numbers of beetles every year in areas that we visited each year between 2012 and 2016.

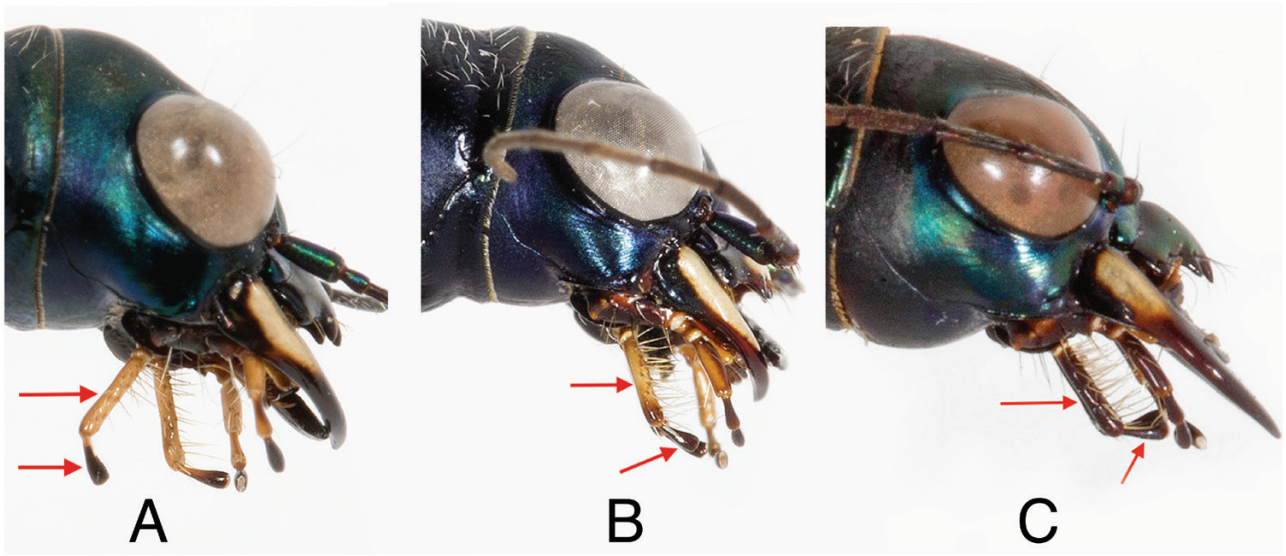

Figure 7. Labial palps of: A, *D. pruininus*; B, *D. belfragei*; C, ***D. knisleyi* sp. nov.** A and B illustrate labial palps with a contrasting darker apical segment, and C illustrates labial palps that are consistently dark throughout.

KEY TO THE GENUS *DROMOCHORUS*

1a. Labial palps at least partly yellow to dark amber, with contrasting darker apical segment (Fig. 7A, B) 2
1b. Labial palps consistently dark brown to black throughout (Fig. 7C) 4
2a. Elytral surface smooth and finely frosted in texture, without any pitting or subsutural foveae. Dorsum with strong blue to violet reflections throughout. Labial palps yellow, with contrasting darker apical segment. Kansas and western Missouri, south to Central Texas ..... *D. pruininus*
2b. Elytra surface dull, textured with fine to deep pitting throughout. Distinct shallow pits running along elytra suture (subsutural foveae, Fig. 8A) may be present 3
3a. Dorsum dark brown with prominent shallow subsutural foveae and irregular pitting throughout elytral surface. Foveae and smaller pits with metallic green reflections. Irregular green marbling may be present on elytra, head and pronotum. Labial palps yellow, with contrasting darker apical segment. Louisiana to East Texas ..... *D. pilatei*
3b. Dorsum black with shallow to deep pitting on elytral surface. Some metallic blue reflections may be present, especially on the supraorbital region of the head and humeral area of the elytra. Subsutural foveae may be present. Labial palps yellow to dark amber, with apical segment darkest. Oklahoma and Texas panhandle, south to East Central Texas ..... *D. belfragei*
4a. Elytral surface rough, with shallow to deep pitting. Subsutural foveae present (Fig. 8A), often with metallic green or blue reflections in pits. 'Hill Country' region of Central Texas..... *D. knisleyi* sp. nov.
4b. Elytral surface smooth, often with velvety or frosted texture. No subsutural foveae present (Fig. 8B) 5
5a. Pronotum glabrous or with few scattered long thin erect setae irregularly placed, rarely concentrated along margins 6
5b. Pronotum with sparse to regular white decumbent setae, mostly in lateral third 7
6a. Dorsum finely velvety black, with strong violet, blue or green reflections, especially along margins. Male labrum entirely dark or very nearly so. Body form gracile. South Texas, coastal areas south of Corpus Christi and inland to vicinity of Dimmit County ..... *D. velutinigrens*
6b. Elytra dark ash-grey, sometimes with frosty blue reflections. South Texas in mesquite chaparral forest. Known only from Bexar, Frio and Atascosa Counties ..... *D. minimus* sp. nov.
7a. Elytra dull black, may have bluish reflections especially near margins. South Texas to Mexico, in mesquite chaparral. Known from Dimmit, LaSalle, and Webb Counties in Texas, and the state of Tamaulipas, Mexico ..... *D. chaparralensis* sp. nov.
7b. Elytra finely velvety black, may have a faint dark blue sheen. Found in coastal prairie habitat near Gulf of Mexico ..... *D. welderensis* sp. nov.

DROMOCHORUS PRUININUS CASEY, 1897 (FIGS 7A, 9A)

Common name
Frosted tiger beetle.

Type locality
'Kansas'. Syntypes (3) in USNM, Washington DC (Fig. 9A; map Fig. 3).

Synonymy
Cicindela pruinina Horn, 1915.
Dromochorus pruinius Johnson, 1991 (unjustified emendation, misspelling).

Taxonomic history
Dromochorus pruininus was described by Casey (1897) as a species, but its taxonomic status has been contentious in recent decades. Freitag (1999) regarded *D. pruininus* as conspecific with *D. belfragei*. Pearson *et al.* (2006) acknowledged the uncertainty of its placement and assessed that *D. pruininus* could be either a 'form' or subspecies of *D. belfragei*, or possibly a full species. Other workers have regarded it as a distinct species (e.g. Johnson, 1991; Wiesner, 1992; Bousquet, 2012). Due to the relative paucity of specimens in most museums, previous assessments of species status were necessarily made with limited morphological and geographical data. Here we recognize *D. pruininus* as a distinct species from *D. belfragei* and all others, based on reciprocal monophyly (mtDNA) (Fig. 2), multiple diagnostic morphological characteristics and ecological characteristics.

Distribution
Dromochorus pruininus is the northernmost species in the genus, occurring from Missouri, Kansas and

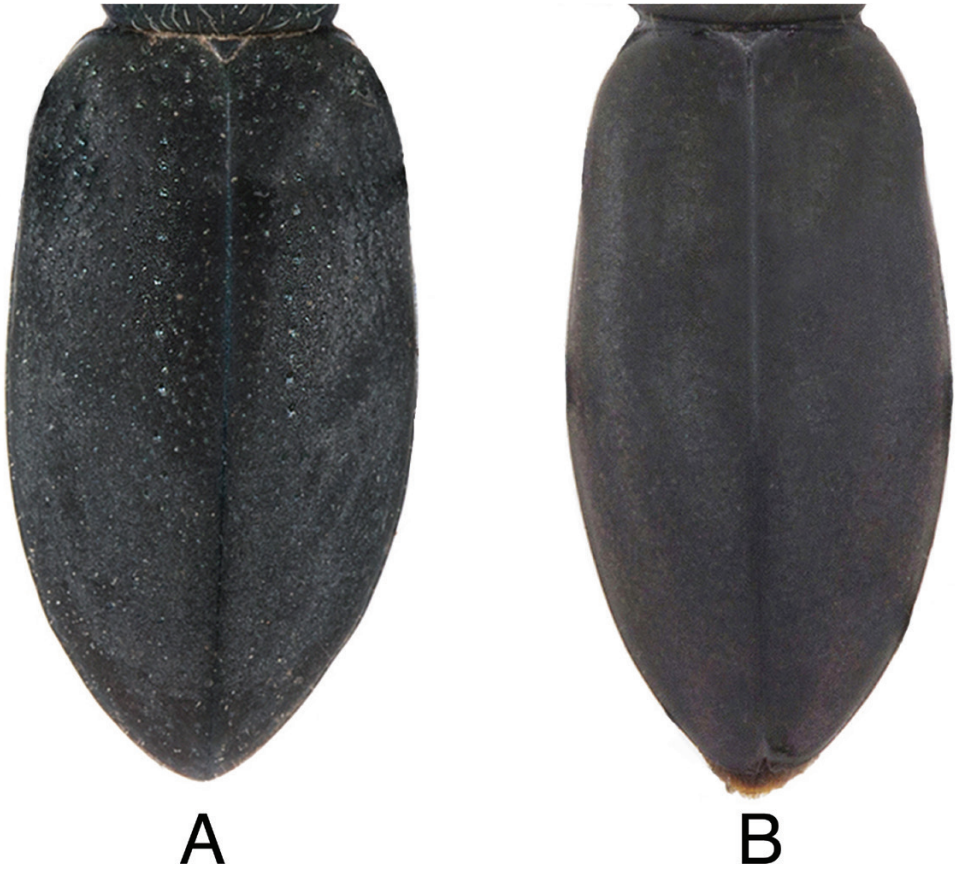

Figure 8. A, Elytra showing distinct punctures (foveae) running parallel to the suture of the elytra (***D. knisleyi* sp. nov.**). B, lack of distinct subsutural foveae (***D. welderensis* sp. nov.**).

Oklahoma to Central Texas. It appears to be allopatric with *D. belfragei*, however, as the two ranges come in close proximity in several areas (Fig. 3). Despite the near geographic overlap of the two taxa, there was no evidence of interbreeding based on in the mtDNA genealogy (Fig. 2B).

Diagnosis

This species is distinctive, and can be distinguished from all other *Dromochorus* by the presence of a frosted, dark-blue dorsum, in conjunction with maxillary palpi that are yellow-ochre with a contrasting dark apical segment (Fig. 7). *Dromochorus pruininus* also lacks any elytral pitting, subsutural foveae or dark infuscations on the elytra.

Description

Large-sized *Dromochorus*. Body length 12.3–14.6 mm, mean ♀ 14.0 mm, mean ♂ 13.1 mm. Head slightly wider than pronotum. **Head black with frosted blue and/or violet sheen**. Fine rugosity often present on the frons and vertex. All head portions glabrous, except for two supraorbital setae next to each eye. Frons concave in median area, especially in male, bulging towards slightly convex near the anterior margin, clearly delimited from clypeus, gradually blending into vertex. Genae bright polished metallic blue or green, blending to violet posteriorly, with shallow longitudinal striae gradually ending at border of vertex. Clypeus shimmering blue, occasionally with violet reflections along the margins. Male labrum tridentate with 6–8 setae, central area pale ochre-testaceous, with a thin, dark-brown to black border anteriorly and posteriorly, dark brown to black laterally; female labrum tridentate with 6–8 setae, entirely dark brown to black with polished metallic cupreous to green reflections. **Maxillary and labial palpi yellow-ochre to pale amber; apical segment dark brown to black**, often with metallic purple and green reflections. Antennae normal length, reaching back to humerus and basal third of elytron, slightly longer in male than female; scape dark testaceous to black with metallic reflections of violet, cupreous and green, with 2–3 apical setae; pedicel dark testaceous with metallic reflections of violet, cupreous and green, lacking any setae; flagellum dark testaceous, antennomeres 3–4 with metallic violet and green reflections, densely clothed in short

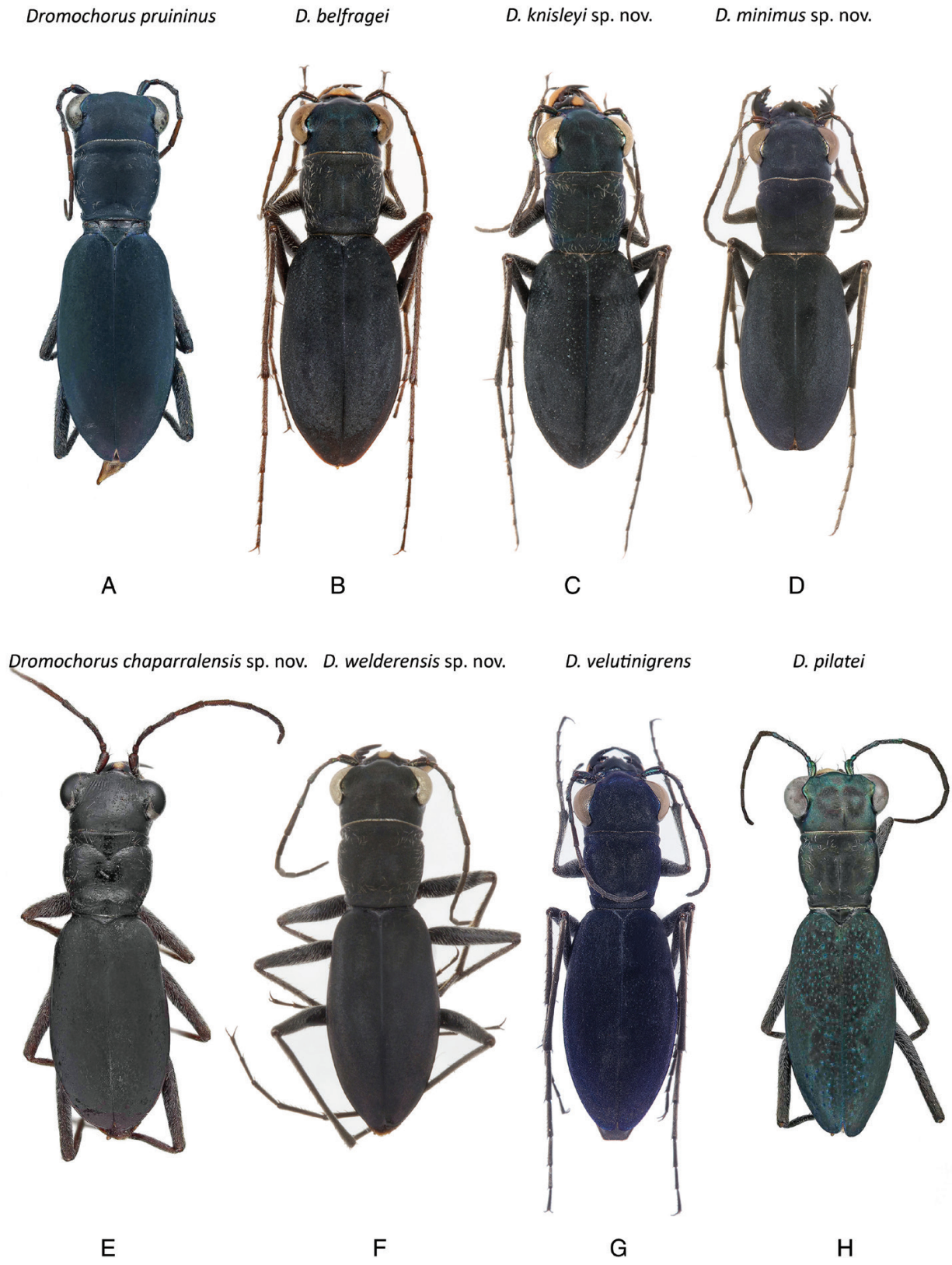

Figure 9. Dorsal habitus for species of *Dromochorus*. A, *D. pruininus*; B, *D. belfragei*; C, ***D. knisleyi* sp. nov.**; D, ***D. minimus* sp. nov.**; E, ***D. chaparralensis* sp. nov.**; F, ***D. welderensis* sp. nov.**; G, *D. velutinigrens*; H, *D. pilatei*.

white setae, antennomeres 5–11 dull-textured without metallic reflections and possessing erect setae in apical rings only, covered with fine pubescence throughout.

Thorax: Pronotum 2.4–3.3 mm in length, mean ♀ 2.9 mm, mean ♂ 2.8 mm; width 2.8–3.4 mm, mean ♀ 3.2 mm, mean ♂ 3.1 mm. Pronotum black, with dark-blue, frosted surface, slightly wider than long, widest near anterior margin, width to length ratio 1.0 to 1.3, setae sparse and irregular, mostly present along lateral third of dorsal surface; disc finely rugose, with thin but distinct median line, with less well-defined shallow sulci present anteriorly and posteriorly; notopleural sutures clearly defined, not visible from dorsal view; proepisternum black with weak to strong iridescent blue reflections, glabrous. Elytra elongate, convex, 7.5–9.3 mm length, mean ♀ 8.5 mm, mean ♂ 8.0 mm, shape similar in both sexes, but slightly wider in female, especially toward apical third; sutural spine absent, microserrations not present on elytral apices; **elytral texture dull throughout, elytral coloration black with frosted blue to violet sheen; maculations absent; infuscations absent; subsutural foveae absent**.

Legs: Pro-, meso- and metacoxae dark testaceous to black with iridescent blue to violet and cupreous reflections, with numerous setae; pro- and mesotrochanters with a single erect seta, metatrochanter glabrous, trochanters dark brown-testaceous; femora metallic green to violet, densely clothed in decumbent white setae; tibiae brown, clothed with setae of two types: sparser brown-testaceous long setae and dense short decumbent white setae; two tibial spines present; tarsi brown-testaceous, first three dilated protarsomeres in male with dense greyish-white setal pad.

Abdomen: Venter black with metallic violet to greenish reflections throughout most surfaces. Decumbent white setae present on ventrite 1. Ventrites 2–6 have scattered short brown recumbent setae present throughout, but often abraded.

Ecology / natural history

Dromochorus pruininus appears to have a 2-year life cycle based on observations from our Dallas-Fort Worth (DFW) area study sites. Adults are active between mid-May and early July in the southern part of its range (e.g. central to north TX) and late June to early August in northern part of its range (e.g. north-west OK to KS). Based on detailed observations of populations in the DFW area, peak adult activity is approximately 3–4 weeks after initial emergence.

Dromochorus pruininus is mostly associated with riparian systems with strong clay content. However, it is rarely found on muddy streambanks, but instead is typically found on higher elevation or relief within the riparian system. The species also occurs in sodded and cultivated fields, hill-tops, road-side ditches and meadows (Larochelle & Larivière, 2001; Pearson *et al.*, 2006). *Dromochorus pruininus* appears to be most associated with clay soils that exhibit large cracks when dry, and beetles have been observed using these cracks to escape. Although it has been considered a habitat generalist (Larochelle & Larivière, 2001), proximal optimal oviposition habitat is likely involved in determining where adults are most active, and this observation is reinforced by the majority of our observations of adults tending to be concentrated near larval burrow sites. The eurytopic (able to tolerate a wide range of habitats) point of view is probably due to a lack of understanding regarding the preferred microhabitat association of *D. pruininus*, coupled with the fact that this species cannot fly and must walk through these areas of suboptimal habitat to disperse. Although adult *D. pruininus* must move through thick vegetation, this species requires semi-open areas for mating, foraging and oviposition. Like all other *Dromochorus*, *D. pruininus* will run into dense grasses to hide when pursued.

Various accounts have been reported regarding daily activity for the adults. They are thought to be most active in early morning and afternoon/evening (Pearson *et al.*, 2015), with most activity in late afternoon/early evening (MacRae & Brown, 2011) or active after 4pm (Larochelle & Larivière, 2001). Between 2012 and 2016, the authors have observed the species active at every hour of daylight. Most activity occurs in the 2–3 h after sunrise, and in the 2–3 h before dark, but when there is significant cloud cover, beetles may be present at any time of the day. In addition, shaded microhabitats may permit beetles to be more active than in nearby open microhabitats, suggesting that adults are more photophobic than crepuscular. Despite a tendency to generally avoid strong sunlight, our observations indicate that this species appears to tolerate direct sun much more than *D. belfragei*. *Dromochorus pruininus* are not active at night, and no beetles were ever observed at light traps, even those that were placed at sites where adults were observed during the same day.

At one DFW area study site, larval burrows were observed in a variety of open areas and vegetated areas, especially along loose soil berms, small mammalian trails that cut through heavy vegetation along steep/eroding clay banks, cracked clay areas with heavy grasses along upper banks, freshly disturbed areas (e.g. tracks from off-road vehicles that

cut through grasses), the middle and edges of open trails, flat areas near the water's edge of rivers, the base of ant mounds, clay areas with >50% grassy cover and the base of mesquite trees in riparian zones. Females seem to prefer crusty or loose clay for oviposition, even in areas with extensive vegetation (above 150 cm in height in late summer). Spomer *et al.* (2008) demonstrated that in a laboratory setting this species showed a preference for oviposition along sloped surfaces; however, we have observed larval burrows in flat, level areas, as often as sloped, in the field. In the autumn, burrows have been found in heavily shaded and soggy areas along riparian wooded middle banks under clover.

Pearson *et al.* (2006) suggest that *D. pruininus/belfragei* have been impacted by the introduction of the red imported fire ant, *Solenopsis invicta,* and report that *Dromochorus* has all but disappeared in certain areas. Our observations do not support this hypothesis. In our fieldwork, we found *D. pruininus* sympatric with *S. invicta* at three sites in the DFW area. *S. invicta* is abundant at two of the sites and at lower densities at the third. The distribution of *S. invicta* extended to sites within Dallas and Tarrant Counties as early 1958–67 (Cokendolpher & Phillips, 1989), and we have monitored beetles at the sites between 2012 and 2014. We observed that *D. pruininus* are abundant near fire ant mounds, with >81 beetles seen per hour at peak activity (Herrmann, Duran & Egan, unpublished). Moreover, using *S. invicta*-specific mtDNA markers, we screened the gut contents of hundreds of beetles, and found that ~70% had recently fed on fire ants (Duran, Herrmann & Egan, unpublished). All data to date suggest that *D. pruininus*, and likely all other *Dromochorus*, are not displaced by fire ants and, moreover, they appear to frequently prey upon them.

Third instar larvae are commonly parasitized with *Anthrax* sp. larvae at the DFW study site.

DROMOCHORUS BELFRAGEI SALLÉ, 1877 (FIGS 7B, 9B)

Common name
Loamy-ground tiger beetle.

Type locality
'Texas–Dallas, Wasco (presumed to be a misspelling of Waco), etc., on the banks of the Trinity River' (English translation). Syntypes unknown, probably in MHNP (Bousquet 2012).

Synonymy
Dromochorus bellefragei Heyne, 1893 (unjustified emendation).

Dromochorus sericeus Casey, 1897: 294.

Type locality
'Texas'. Two syntypes in USNM, Washington DC (synonymy established by Leng, 1902).

Taxonomic history
Dromochorus belfragei has been a catch-all taxon, and many populations of *Dromochorus* were lumped under this name in the literature or in museum collections prior to this revision. *Dromochorus pilatei* and *D. velutinigrens* are the only nominal taxa that have never been considered conspecific with *D. belfragei*. Based on morphology, ecology, biogeography, mtDNA genealogy and multilocus genetic data, *D. belfragei* (*sensu stricto*) is circumscribed as a separate species from three other distinct species, *D. pruininus*, *D. knisleyi* **sp. nov.** and *D. minimus* **sp. nov.** Occasional hybridization with *D. knisleyi* has been observed where their ranges come in contact, and this is further supported by evidence of mtDNA introgression.

Distribution
Dromochorus belfragei is known to occur from the north-western panhandle of Texas and southern Oklahoma to south-eastern Texas. Previous literature described a more extensive range from south-eastern Colorado (Michels *et al.*, 2008) to Tamaulipas, Mexico (Cazier, 1954). The former record now appears to be an error (Michels, pers. comm., 2015), and the latter would appear to belong to *D. chaparralensis* **sp. nov.** One specimen from the AMNH was labelled 'St. George, Utah', although this locality would not appear plausible. The range of *D. belfragei* comes close to several other species. In particular, *D. belfragei* is nearly sympatric with *D. pruininus* in several places (Fig. 3; see *D. pruininus* account). In eastern and southern Bexar County, TX, this species is found sympatrically with *D. knisleyi*, and possibly *D. minimus*.

Diagnosis
This species can be distinguished from all other similar *Dromochorus* by the presence of finely to coarsely pitted black elytra that are not frosted in texture, in conjunction with maxillary palpi that have the apical segment darkest, with dark yellow-ochre to dark amber coloration in other segments (Fig. 8). Most populations of *D. belfragei* possess at least small subsutural foveae, but these lack metallic reflections. Southern and eastern populations may have subtle dark infuscations on the elytra.

Dromochorus belfragei is most likely to be confused with *D. pruininus*, *knisleyi* or *welderensis*. It can be separated from these taxa by the following:

1. *Dromochorus pruininus* has a frosted blue sheen on the elytra and the entire dorsal surface, and it

lacks any pits or subsutural foveae. The maxillary palpi in *D. pruininus* are pale yellow-ochre with a dark apical segment, whereas *D. belfragei* has darker yellow-red to red testaceous palpi with a dark apical segment.

2. *Dromochorus knisleyi* is similar to *D. belfragei*, but has more prominent subsutural foveae, often with bright metallic blue, green or gold reflections. In *D. knisleyi*, the maxillary palpi are always dark in all segments, whereas in *D. belfragei*, the apical segment is darker than the preceding. Infuscations are always present in *D. knisleyi* and are usually absent in *D. belfragei*. Ecologically, *D. knisleyi* is found in upland juniper woodland in the Edwards Plateau, and *D. belfragei* is found outside of this region, in clay soils associated with larger riparian systems. The two species appear to hybridize along a narrow contact zone at the edge of the Balcones Escarpment in south-eastern and south-central Texas. Both species and their hybrids may be found in this area. The existence of a hybrid zone was further supported by mtDNA data (Fig. 2).
3. *Dromochorus welderensis* has a smooth velvety black elytral surface (may have metallic blue-violet reflections), and never has pitting or subsutural foveae. The maxillary palpi are always dark in all segments, whereas in *D. belfragei*, the apical segment is darker than the preceding.

Description

Medium- to large-sized *Dromochorus*. Body length 11.6–15.2 mm, mean ♀ 14.5 mm, mean ♂ 13.2 mm. Head slightly wider than pronotum. **Head predominantly black with blue reflections mostly concentrated near the anterior margin and edges of the supraorbital region**. Fine rugosity often present on the frons and vertex. All head portions glabrous except for two supraorbital setae next to each eye. Frons concave in median area, especially in male, bulging towards slightly convex near anterior margin, clearly delimited from clypeus, gradually blending into vertex. Genae bright polished metallic violet to blue, with shallow, longitudinal striae gradually ending at border of vertex. Clypeus mostly black, with patches of metallic blue or violet reflections; clypeus may be nearly entirely blue to violet in females. Male labrum tridentate with 6–8 setae, central area pale ochre-testaceous, with a thin dark-brown to black border posteriorly and sometimes anteriorly, dark-brown to black laterally; in some populations, the pale central area of the labrum may exist as a small spot, in others the pale area may cover more than two-thirds of the total labrum surface; female labrum tridentate with 6–8 setae, entirely dark-brown to black with polished metallic cupreous to green reflections. **Maxillary and labial palpi with apical segment darker than other segments; basal to penultimate segments yellow-ochre to dark red-amber**, often with metallic purple and green reflections. Antennae normal length, reaching back to humerus and basal third of elytron, slightly longer in male than female; scape dark testaceous to black with metallic reflections of violet, cupreous and green, with 2–3 apical setae; pedicel dark testaceous with metallic reflections of violet, cupreous and green, lacking any setae; flagellum dark testaceous, antennomeres 3–4 with metallic violet and green reflections, densely clothed in short white setae, antennomeres 5–11 dull-textured without metallic reflections and possessing erect setae in apical rings only, covered with fine pubescence throughout.

Thorax: Pronotum 2.5–3.4 mm in length, mean ♀ 3.1 mm, mean ♂ 2.8 mm; width 2.8–3.6 mm, mean ♀ 3.4 mm, mean ♂ 3.1 mm. Pronotum black, with some dark-blue reflections, especially in sulci, slightly wider than long, widest near anterior margin, width to length ratio 1.0 to 1.2, setae sparse to regularly spaced, mostly present along lateral third of dorsal surface; disc finely rugose, with thin but distinct median line, with well-defined shallow sulci present anteriorly and posteriorly; notopleural sutures clearly defined, not visible from dorsal view; proepisternum black with weak to strong iridescent blue reflections, glabrous. Elytra convex, elongate, 7.1–9.7 mm length, mean ♀ 8.8 mm, mean ♂ 8.2 mm, shape similar in both sexes, but slightly wider in female, especially toward apical third; sutural spine absent, microserrations not present on elytral apices; **elytral texture dull, with regular small pits present throughout disk, elytral coloration black, often with blue reflections near humeral region; elytral maculations absent; infuscations rarely present; subsutural foveae, when present, only slightly more prominent than other pits on the elytral disk; subsutural foveae lacking bright metallic reflections, except rarely in basal area**.

Legs: Pro-, meso- and metacoxae dark testaceous to black with iridescent blue to violet and cupreous reflections, with numerous setae; pro- and meso- trochanters with a single erect seta, metatrochanter glabrous, trochanters dark brown-testaceous, dark brown-testaceous; femora black with metallic violet and green reflections, densely clothed in decumbent white setae; tibiae testaceous brown, clothed with setae of two types: sparser brown-testaceous long setae and dense short decumbent white setae; two tibial spines present; tarsi brown-testaceous, first three dilated protarsomeres in male with dense greyish-white setal pad.

Abdomen: Venter mostly black with occasional metallic violet reflections. Decumbent white setae present on ventrite 1. Ventrites 2–6 have sparse, short, brown erect setae present throughout, but often abraded.

Ecology / natural history
Dromochorus belfragei adults are active between mid-May and early July in most of its range (e.g. central to north TX) and late June to late July in northern part of its range (e.g. OK to panhandle of TX).

Dromochorus belfragei can be found in natural and managed forested and agricultural areas (e.g. pecan groves) that have semi-open shaded areas or trails beneath the canopy. Soils in these areas can be dark, red to black in color, clay to clay-loam, cracked and sometimes moist in low areas that are heavily trampled by cattle. Adult beetles tend to avoid the lighter coloured sandy areas that are exposed to full sunlight on the forest edges. Despite the common name, this species appears more closely associated with soils that possess high clay content, more so than loam. The preferred habitat of *D. belfragei* is generally associated with larger riparian systems, although the beetles are not typically found near the water's edge and we have found them over 3 km from water. They appear to have a wider ecological niche than most *Dromochorus*; their habitat and geographic distribution encompass a larger number of ecoregions than other species (Fig. 3; Table 1).

Dromochorus belfragei and *D. pruininus* have similar but non-overlapping ranges, and they may be separated by ecological barriers, at least in some areas. In the DFW area of North Texas, the species has been reported a few kilometers from *D. pruininus* (Pearson *et al.*, 2006), but the two appear to be separated by a narrow extension of the EPA Level IV Eastern Cross Timbers ecoregion, which is not suitable for either species. The forested undergrowth of this area is extremely dense in many areas, and may not possess the necessary surface soil conditions for either species to persist. *Dromochorus belfragei* may be more susceptible to urbanization than *D. pruininus*, as none have been collected in the DFW area (Tarrant Co.) since the 1970s, whereas *D. pruininus* appear much more tolerant to these disturbances and may be abundant in semi-open grassy areas where trails have been established in riparian parks (Dallas, Collin Co.).

Thought to be crepuscular, this species is active throughout the day in shady areas or when overcast. Similar in behaviour to *D. pruininus* and *D. pilatei*, this species has been observed using soil cracks for escape, especially during dry conditions when virtisolic cracks are pronounced. Like other *Dromochorus*, *D. belfragei* frequently moves to vegetated cover to escape when pursued.

DROMOCHORUS KNISLEYI DURAN, HERRMANN, ROMAN & EGAN **SP. NOV.**

(FIGS 7C, 8A, 9C)

Common name
Juniper grove tiger beetle.

Type locality
Vicinity of Pedernales Falls, Texas. Holotype (USNM): 1 ♂, USA: Texas: Blanco Co./Vicinity of Pedernales/19-VI-2013/leg D. Duran. Paratypes: 14 ♂♂, 19 ♀♀, USA: Texas: Blanco Co./Vicinity of Pedernales Falls St. Pk./19-VI-2013/leg D. Duran. 6 ♂♂, 7 ♀♀, USA: Texas: Blanco Co./Vicinity of Pedernales Falls St. Pk./08-VI-2015/leg S.J Roman. 5 ♂♂, 3 ♀♀, USA: Texas: Blanco Co./Vicinity of Pedernales Falls St. Pk./01-VI-2014/leg D. Brzoska. 2 ♂, USA: Texas: Blanco Co./Park E. of Pedernales Falls S.P./20-VI-2014/leg D. Duran. 2 ♀, USA: TEXAS: Bandera Co./3 miles W of Pipe Creek/22-VI-2013/leg D. Duran.

Distribution
This species is only known from the Edwards Plateau of central Texas, locally known as the Texas Hill Country. It comes in close geographic proximity to *D. belfragei* and *D. minimus*, where all three species distributions converge at the edge of the Balcones Escarpment in Bexar County. Analyses of mtDNA data indicate that hybridization occurs in this contact zone, and apparent hybrid *D. knisleyi* x *belfragei* individuals have been found in this area.

Diagnosis
Dromochorus knisleyi is most easily confused with the sister taxa *D. belfragei*. For differential diagnosis, see *D. belfragei* species account.

Description
Medium-sized *Dromochorus*. Body length 10.9–14.4 mm, mean ♀ 13.0 mm, mean ♂ 12.2 mm. Head slightly wider than pronotum. **Head predominantly charcoal black with blue reflections mostly concentrated near the anterior margin and edges of the supraorbital region**. Fine rugosity often present on the frons and vertex. All head portions glabrous except for two supraorbital setae next to each eye. Frons concave in median area, especially in male, bulging towards slightly convex near anterior margin, clearly delimited from clypeus, gradually blending into vertex. Genae black or bright polished metallic violet to blue, with shallow longitudinal striae gradually ending at border of vertex. Clypeus bronze with green to blue reflections throughout. Male labrum tridentate with 6–8 setae, central area pale ochre-testaceous, with a thin dark-brown to black border posteriorly and sometimes anteriorly, dark-brown to black laterally; the

pale central area of the labrum may exist as a small spot, up to one-third of the total labrum surface; female labrum tridentate with 6–8 setae, entirely dark-brown to black with polished metallic cupreous to green reflections. **All segments of maxillary and labial palpi consistently dark-brown; apical segment is not darker than other segments**. Antennae normal length, reaching back to humerus and basal third of elytron, slightly longer in male than female; scape dark testaceous to black with metallic reflections of violet, cupreous and green, with 2–3 apical setae; pedicel dark testaceous with metallic reflections of violet, cupreous and green, lacking any setae; flagellum dark testaceous, antennomeres 3–4 with metallic violet and green reflections, densely clothed in short white setae, antennomeres 5–11 dull-textured without metallic reflections and possessing erect setae in apical rings only, covered with fine pubescence throughout.

Thorax: Pronotum 2.4–3.2 mm in length, mean ♀ 2.9 mm, mean ♂ 2.8 mm; width 2.5–3.2 mm, mean ♀ 3.0 mm, mean ♂ 2.9 mm. Pronotum charcoal black, with green to blue or violet reflections, especially along lateral margins, slightly wider than long, widest near anterior margin, width to length ratio 0.9 to 1.1, setae sparse to regularly spaced, mostly present along lateral third of dorsal surface; disc finely rugose, with thin but distinct median line, with well-defined shallow sulci present anteriorly and posteriorly; notopleural sutures clearly defined, not visible from dorsal view; proepisternum black with weak to strong iridescent blue to violet reflections, glabrous. Elytra elongate, 6.8–8.7 mm length, mean ♀ 7.9 mm, mean ♂ 7.7 mm, shape similar in both sexes, but slightly wider in female, especially toward apical third; sutural spine absent, microserrations not present on elytral apices; elytral dorsal surface convex; **elytral texture dull, with regular small pits present throughout disk, elytral coloration charcoal black, often with blue reflections near humeral region**; elytral maculations absent; **two dark oblique infuscations present; subsutural foveae prominent, typically with metallic blue, green, or gold reflections**.

Legs: Pro-, meso-, and metacoxae dark testaceous to black with iridescent blue to violet and cupreous reflections, sparse setae on pro- and mesocoxae, fewer on metacoxae; pro- and mesotrochanters with a single erect seta, metatrochanter glabrous, trochanters dark brown-testaceous; femora black with metallic violet and green reflections, densely clothed in decumbent white setae; tibiae testaceous brown, clothed with setae of two types: sparser brown-testaceous long setae and dense short decumbent white setae; two tibial spines present; tarsi brown-testaceous, first three dilated protarsomeres in male with dense greyish-white setal pad.

Abdomen: Venter mostly black with occasional metallic green to violet reflections. Decumbent white setae present on ventrite 1. Ventrites 2–6 have sparse short brown erect setae present throughout, but often abraded.

Etymology

Named for Dr C. Barry Knisley, one of the leading authorities on North American tiger beetle conservation and ecology. D.P. Duran and R.A. Gwiazdowski are greatly indebted to Barry for his mentorship and friendship.

Ecology / natural history

Dromochorus knisleyi adults have been found from mid-May to late June, but it is likely that they could be active outside of this window.

Dromochorus knisleyi is found in upland juniper-oak woodlands in the Edwards Plateau, and does not appear to be strongly associated with riparian areas. The preferred habitat is late succession stands of juniper and, as such, it can be difficult for a collector to easily walk through these areas. Adult beetles are active throughout the day and are present in semi-open grassy areas under the cover of juniper trees. The first author observed dozens of beetles over a span of two days, and all adult activity was restricted to these forested areas. Beetles foraged and mated exclusively near or under juniper boughs. Moreover, even during cloudy periods and late in the afternoon, none were observed moving into more open grassy areas outside of the juniper stands. Moreover, beetles were not present in woodlands dominated by oaks. In mixed juniper–oak woodlands, beetles were found exclusively near junipers. This species may be the most ecologically specialized of all *Dromochorus*.

More observations are needed for this rarely collected species. Many aspects of the biology are currently unknown.

DROMOCHORUS MINIMUS DURAN, ROMAN, HERRMANN & EGAN **SP. NOV.**

(FIG. 9D)

Common name

Pygmy dromo tiger beetle.

Type locality

SE of Pleasanton, TX. Holotype (deposited in USNM): 1 ♂, USA: Texas: Atascosa Co./SE of Pleasanton/19-VI-2014/leg D. Duran. Paratypes: 3 ♂♂, 3 ♀♀, USA: Texas: Atascosa Co./SE of Pleasanton/19-VI-2014/leg D. Duran. 3 ♂, 4 ♀♀,

USA: Texas: Atascosa Co./SE. of Pleasanton/30-V-2014/leg D. Sunberg. 1 ♂, 1 ♀, USA: Texas: Atascosa Co./SE. of Pleasanton/02-VI-2015/leg S.J. Roman. 17 ♂♂, 7 ♀♀, USA: Texas: Atascosa Co./SE. of Pleasanton/29-VI-2014/leg D. Brzoska. 1 ♂, USA, Texas: Bexar Co./7 miles S. San Antonio/06-VI-2010/leg G. Waldren. 1 ♂, USA: Texas: Bexar Co./Loop 1604 Hwy/12-VI-2016/leg J. Back.

Distribution

Central/south Texas, south of the Balcones Escarpment. Currently known only from Bexar, Atascosa and Frio Counties.

Diagnosis

Dromochorus minimus can be separated from all other species, by the presence of a frosted or ashy grey to beige dorsum, sometimes with blue reflections, in conjunction with labial palpi that are all dark (apical segment is not darker than other segments), and sparse erect setae on the pronotum, often irregularly placed throughout.

This species is most likely to be confused with *D. pruininus*, *chaparralensis* or *welderensis*.

Dromochorus pruininus is generally larger (Fig. 6; Table 2), has pale maxillary palps with a contrasting dark apical segment and is also separable by geographic range.

Dromochorus chaparralensis is usually larger (Fig. 6; Table 2), and lacks any prominent frosted texturing on the dorsal surface. The pronotum of *D. chaparralensis* has setae more regularly arranged, mostly along lateral third.

Dromochorus welderensis is usually larger (Fig. 6; Table 2), and its pronotum has decumbent white setae, mostly along lateral third. The habitat of *D. welderensis* is Gulf Coast Prairie.

Description

Small- to medium-sized *Dromochorus*. Body length 10.5–13.7 mm, mean ♀ 12.7 mm, mean ♂ 11.7 mm. Head slightly wider than pronotum. Head predominantly charcoal black with blue to green reflections mostly concentrated near the anterior margin and edges of the supraorbital region. Fine rugosity often present on the frons and vertex. All head portions glabrous except for two supraorbital setae next to each eye. Frons concave in median area, especially in male, bulging towards slightly convex near anterior margin, clearly delimited from clypeus, gradually blending into vertex. Genae black with bright polished metallic green to violet reflections, with shallow longitudinal striae gradually ending at border of vertex. Clypeus shining black with blue to violet reflections throughout. Male labrum tridentate with 6–8 setae, central area pale ochre-testaceous, with a thin dark-brown to black border posteriorly and sometimes anteriorly, dark-brown to black laterally; in some populations, the pale central area of the labrum may exist as a small spot, up to one-third of the total labrum surface; female labrum tridentate with 6–8 setae, entirely dark brown to black with polished metallic cupreous to green reflections. **All segments of maxillary and labial palpi consistently dark-brown; apical segment is not darker than other segments**. Antennae normal length, reaching back to humerus and basal third of elytron, slightly longer in male than female; scape dark testaceous to black with metallic reflections of violet, cupreous and green, with 2–3 apical setae; pedicel dark testaceous with metallic reflections of violet, cupreous and green, lacking any setae; flagellum dark testaceous, antennomeres 3–4 with metallic violet and green reflections, densely clothed in short white setae, antennomeres 5–11 dull-textured without metallic reflections and possessing erect setae in apical rings only, covered with fine pubescence throughout.

Thorax: Pronotum 2.4–3.1 mm in length, mean ♀ 2.8 mm, mean ♂ 2.7 mm; width 2.5–3.3 mm, mean ♀ 3.0 mm, mean ♂ 2.7 mm. Pronotum charcoal black, typically with frosty pale grey to brown, or blue to violet sheen, especially along lateral margins, slightly wider than long, widest near anterior margin, width to length ratio 1.0 to 1.1, **thin erect setae sparse to irregularly spaced on pronotum**; disc finely rugose, with thin but distinct median line, with well-defined shallow sulci present anteriorly and posteriorly; notopleural sutures clearly defined, not visible from dorsal view; proepisternum black with iridescent olive green to violet reflections, glabrous. Elytra elongate, dorsal surface convex, 6.4–8.0 mm length, mean ♀ 7.6 mm, mean ♂ 7.1 mm, shape similar in both sexes, but slightly wider in female, especially toward apical third; sutural spine absent, microserrations not present on elytral apices; elytral texture dull, with no pitting present, **elytral coloration charcoal black, typically with grey, brown or blue-grey frosted texture along lateral margins, apex with blue or grey frosted texture**; elytral maculations absent; **subsutural foveae absent**.

Legs: Pro-, meso- and metacoxae dark brown with iridescent violet and cupreous reflections, numerous setae on pro- and mesocoxae, sparse on metacoxae; pro- and mesotrochanters with a single erect seta, metatrochanter glabrous, trochanters dark brown-testaceous; femora black with metallic violet and green reflections, densely clothed in decumbent white setae; tibiae testaceous brown, clothed with setae of two types: sparser brown-testaceous long setae and dense short decumbent white setae; two tibial spines present; tarsi brown-testaceous, first three dilated protarsomeres in male with dense greyish-white setal pad.

Abdomen: Venter black with metallic olive green and violet reflections. Decumbent white setae present on ventrite 1. Ventrites 2–6 have sparse short brown erect setae present throughout, but often abraded.

Etymology
Dromochorus minimus is named for its smaller size. On average, this species is the smallest in the genus (Table 2).

Ecology / natural history
Little is known about this species' natural history. Adults have been collected from mid-May until late June, but it is possible that the species may be active outside of this window.

Dromochorus minimus occurs in mesquite-chaparral savannah in central/south Texas, just south of the Edwards Plateau, part of the larger Gulf Coastal Plains physiographic province. It has been found in open grassy areas interspersed between mesquite trees and clumps of *Opuntia* cactus. Adult beetles may be found venturing into the open spaces between clumps of grass, and will rapidly run into vegetation if pursued. *Dromochorus minimus* appears to be remarkably swift, even compared to other species of *Dromochorus*.

In direct sunlight, live specimens appear beige-grey to smoky blue-grey.

DROMOCHORUS CHAPARRALENSIS DURAN, ROMAN, HERRMANN & EGAN **SP. NOV.**

(FIG. 9E)

Common name
Chaparral tiger beetle.

Type locality
Carrizo Springs, TX. Holotype (deposited in NMNH): 1 ♀, Carrizo Spgs/Tex. V-27–32 // E. G. Lindsley Collector // M.A. Cazier/Collection. Paratypes: 3 ♂♂, 5 ♀♀, Carrizo Spgs./Tex VI-12–32 // E.G. Lindsley Collector // A. Nicolay collection 1950. 1 ♀, Mexico, Tamaulipas/Nuevo Laredo/20-VI-2010/leg J. Stamatov. 1 ♀, USA: Texas: LaSalle Co./Chaparral W.M.A./19-VI-2013/E. San Gregario. Fig. 9E, Map Fig. 3

Distribution
Inland South Texas (currently known from Dimmit, LaSalle, and Webb Counties) and Tamaulipas, Mexico. This species has not been well sampled and is likely present in adjacent areas within the Gulf Coastal Plain, especially in northern Mexico. It can occur with *D. velutinigrens* in places where sandy soils mix with the dominant heavier red clays.

Diagnosis
Dromochorus chaparralensis is a robust, dark beetle, and most specimens are dull black with little colour on dorsal or ventral surfaces. Some specimens may have bluish reflections along the margins. It is restricted to mesquite chaparral in South Texas.

This species is most likely to be confused with *D. minimus* or *welderensis*.

Dromochorus mimimus is usually smaller (Fig. 6; Table 2), and possesses a prominent frosted ashy grey, beige, or blue sheen on the dorsal surface; the pronotum has sparse thin erect setae.

Dromochorus welderensis is black with a faint dark blue sheen dorsally, in many individuals. The habitat of *D. welderensis* is Gulf Coast Prairie grasslands, as opposed to the more inland and forested mesquite-chaparral habitat of *D. chaparralensis*.

Description
Medium- to large-sized *Dromochorus*. Body length 12.5–14.5 mm, mean ♀ 13.7 mm, mean ♂ 13.0 mm. Head slightly wider than pronotum. Head black with metallic blue to green reflections mostly limited to the lateral ridge of the supraorbital region. Fine to marked rugosity often present on the frons and vertex. All head portions glabrous except for two supraorbital setae next to each eye. Frons concave in median area, especially in male, bulging towards slightly convex near anterior margin, clearly delimited from clypeus, gradually blending into vertex. Genae black often with weak metallic green to violet reflections, with shallow, longitudinal striae, gradually ending at border of vertex. Clypeus shining black, apparently lacking coloured reflections. Male labrum tridentate with 6–8 setae, central area pale ochre-testaceous, with a thin dark-brown to black border posteriorly and sometimes anteriorly, dark-brown to black laterally; in some populations, the pale central area of the labrum may exist as a small spot, up to one-quarter of the total labrum surface; female labrum tridentate with 6–8 setae, entirely dark-brown to black with polished metallic cupreous to green reflections. **All segments of maxillary and labial palpi consistently dark brown; apical segment is not darker than other segments**. Antennae normal length, reaching back to humerus and basal third of elytron, slightly longer in male than female; scape dark testaceous to black with metallic reflections of violet, cupreous and green, with 2–3 apical setae; pedicel dark testaceous with metallic reflections of violet, cupreous and green, lacking any setae; flagellum dark testaceous, antennomeres 3–4 with metallic violet and green reflections, densely clothed in short white setae, antennomeres 5–11 dull-textured without metallic reflections and possessing erect setae in apical rings only, covered with fine pubescence throughout.

Thorax: Pronotum 2.6–3.4 mm in length, mean ♀ 3.1 mm, mean ♂ 3.1 mm; width 2.9–3.5 mm, mean ♀

3.4 mm, mean ♂ 3.1 mm. Pronotum dull black, slightly wider than long, widest near anterior margin, width to length ratio 1.0 to 1.1, setae sparse, mostly present along lateral third of dorsal surface; disc finely rugose, with thin but distinct median line, with well-defined shallow sulci present anteriorly and posteriorly; notopleural sutures clearly defined, not visible from dorsal view; proepisternum black, lacking prominent metallic coloured reflections, glabrous. Elytra elongate, dorsal surface convex, 7.2–8.6 mm length, mean ♀ 8.2 mm, mean ♂ 7.7 mm, shape similar in both sexes, but slightly wider in female, especially toward apical third; sutural spine absent, microserrations not present on elytral apices; **elytral texture dull, with no pitting present, elytral coloration black, may have weak blue reflections along lateral margins**; elytral maculations absent; **subsutural foveae absent**.

Legs: Pro-, meso- and metacoxae black, without or with minimal metallic reflections, numerous setae on pro- and mesocoxae, sparse on metacoxae; pro- and mesotrochanters with a single erect seta, metatrochanter glabrous, trochanters dark brown-testaceous; femora black with metallic violet reflections, densely clothed in decumbent white setae; tibiae brown, clothed with setae of two types: sparser brown-testaceous long setae and dense short decumbent white setae; two tibial spines present; tarsi brown-testaceous, first three dilated protarsomeres in male with dense greyish-white setal pad.

Abdomen: Venter mostly black with metallic olive green and violet reflections. Decumbent setae present on ventrite 1. Ventrites 2–6 have sparse short brown erect setae present throughout, but often abraded.

Etymology
Named for the dominant mesquite-chaparral plant community found throughout the species range. Also, this species has been collected at Chaparral Wildlife Management Area, in Cotulla, Texas.

Ecology / natural history
Very little is known about this species, as most museum specimens are older, and nothing has been recorded about its ecology. The authors of this study have not observed *D. chaparralensis in situ*.

DROMOCHORUS WELDERENSIS DURAN, HERRMANN, ROMAN & EGAN **SP. NOV.**

(FIGS 8B, 9F)

Common name
Gulf prairie tiger beetle.

Type locality
Welder Wildlife Foundation, Sinton, TX. Holotype (deposited in NMNH): 1 ♂, Texas: San Patricio Co./Welder Wildlife Foundation/11-June-2013/Coll: A. Mitchell. Paratypes: 18 ♂♂, 15 ♀♀, Texas: San Patricio Co./Welder Wildlife Foundation/11-June-2013/Coll: A. Mitchell. 2 ♂, 2 ♀, Texas/Buckeye - Matagorda Co./6-8-17 // J.D. Mitchell collector (NMNH). 2 ♀, Texas: San Patricio Co./Sinton/14-V-1966 // leg. W.T. Murray (JSC). 2 ♂, Texas, Victoria/VI-2–06 // C.R. Jones collector. 1 ♀, Texas: Dickinson/May 29 // TAMU-ENTO X0898573 (TAMUIC).

1 ♂, 1 ♀, Texas: Bee Co./Pettus/10.V.1964 // Leg. Pryor (SFASU). 4 ♂, 1 ♀, Texas: Nueces Co./Luetgens Coll. (AMNH). 1 ♀, Texas: Corpus Christi/VI-7-1969/C.W. Griffin // Nueces River Park (NMNH).

Distribution
Found in the Gulf Prairie ecoregion of coastal Texas, from Houston area to Corpus Christi area.

Diagnosis
Dromochorus welderensis is diagnosable by having a black dorsum, often with a faint dark-blue sheen, and no pitting, subsutural foveae or infuscations, in conjunction with all dark maxillary palps and a pronotum with decumbent white setae.

This species is most likely to be confused with *D. chaparralensis*, *belfragei*, *velutinigrens* or *minimus*.

Dromochorus chaparralensis may be nearly indistinguishable from *D. welderensis* morphologically, but is ecologically differentiated. The habitat of *D. chaparralensis* is forested mesquite-chaparral, unlike the Gulf prairie habitat of *D. welderensis*.

Dromochorus belfragei possesses regular pits on the elytra and often subsutural foveae. Maxillary palpi have a contrasting dark apical segment, with other segments dark yellow-testaceous to dark red-testaceous.

Dromochorus velutinigrens has a very prominent green, blue, or violet dorsal sheen. The body is substantially more narrow and gracile, especially in males. Male *D. velutinigrens* have an all dark labrum, whereas *D. welderensis* males possess a pale central spot. *Dromochorus velutinigrens* have few to no setae on disk of pronotum.

Dromochorus mimimus is generally smaller (Fig. 6; Table 2), and possesses sparse, thin, erect setae on the pronotum. This species also occurs further inland in forested mesquite-chaparral, unlike the Gulf Prairie grassland habitat of *D. welderensis*.

Description
Medium to large-sized *Dromochorus*. Body length 10.9–14.7 mm, mean ♀ 13.7 mm, mean ♂ 12.6 mm. Head slightly wider than pronotum. **Head charcoal brown-black with metallic green, green-blue, or bronze**

reflections mostly limited to the lateral ridge of the supraorbital region. Fine to marked rugosity often present on the frons and vertex. All head portions glabrous except for two supraorbital setae next to each eye. Frons concave in median area, especially in male, bulging towards slightly convex near anterior margin, clearly delimited from clypeus, gradually blending into vertex. Genae black often with metallic green to violet reflections, with shallow longitudinal striae gradually ending at border of vertex. Clypeus black, with metallic violet to green-coloured reflections. Male labrum tridentate with 6–8 setae, central area pale ochre-testaceous, with a thin, dark-brown to black border posteriorly and sometimes anteriorly, dark-brown to black laterally; in some populations, the pale central area of the labrum may exist as a small spot, up to one-quarter of the total labrum surface; female labrum tridentate with 6–8 setae, entirely dark-brown to black with polished metallic cupreous to green reflections. **All segments of maxillary and labial palpi consistently dark-brown; apical segment is not darker than other segments**. Antennae normal length, reaching back to humerus and basal third of elytron, slightly longer in male than female; scape dark testaceous to black with metallic reflections of violet, cupreous and green, with 2–3 apical setae; pedicel dark testaceous with metallic reflections of violet, cupreous and green, lacking any setae; flagellum dark testaceous, antennomeres 3–4 with metallic violet and green reflections, densely clothed in short white setae, antennomeres 5–11 dull-textured without metallic reflections and possessing erect setae in apical rings only, covered with fine pubescence throughout.

Thorax: Pronotum 2.6–3.3 mm in length, mean ♀ 3.1 mm, mean ♂ 2.9 mm; width 2.7–3.4 mm, mean ♀ 3.2 mm, mean ♂ 2.9 mm. Pronotum charcoal brown to black, slightly wider than long, widest near anterior margin, width to length ratio 0.9 to 1.1, setae sparse to regular, mostly present along lateral third of dorsal surface; disc finely rugose, with thin but distinct median line, with well-defined shallow sulci present anteriorly and posteriorly; notopleural sutures clearly defined, not visible from dorsal view; proepisternum black, with metallic violet reflections, glabrous. Elytra elongate, dorsal surface convex, 6.3–8.6 mm length, mean ♀ 8.1 mm, mean ♂ 7.4 mm, shape similar in both sexes, but slightly wider in female, especially toward apical third; sutural spine absent, microserrations not present on elytral apices; **elytral texture dull, with no pitting present, elytral coloration charcoal brown to black, often with faint blue reflections throughout elytral surface**; elytral maculations absent; subsutural foveae absent.

Legs: Pro-, meso- and metacoxae brown to black, with metallic violet to blue reflections, numerous setae on pro- and mesocoxae, sparse on metacoxae; pro- and mesotrochanters with a single erect seta, metatrochanter glabrous, trochanters dark brown-testaceous; femora black with metallic violet reflections, densely clothed in decumbent white setae; tibiae brown, clothed with setae of two types: sparser brown-testaceous long setae and dense, short, decumbent white setae; two tibial spines present; tarsi brown-testaceous, first three dilated protarsomeres in male with dense greyish-white setal pad.

Abdomen: Venter mostly black with metallic olive green and violet reflections. Decumbent setae present on ventrite 1. Ventrites 2–6 have sparse, short, brown erect setae present throughout, but often abraded.

Etymology

Named for the type locality, Welder Wildlife Foundation, in Sinton, Texas, as well as the Foundation's namesake, Robert H. Welder, who established the foundation with the mission to conduct research and education in wildlife management and conservation.

Ecology / natural history

Adults have a long activity period, from mid-May through early August (A. Mitchell, pers. comm.).

Dromochorus welderensis occurs in the Gulf Prairie ecoregion of the Coastal Plain physiographic province of Texas. This *Dromochorus* is the least associated with tree cover, and *D. welderensis* is consistently found in tall grasses in upland prairie habitat. Beetles are often found near heavy clay-loam or clay banks and hills.

This shade-loving species can be observed in early cool mornings or early evenings, and will try to avoid more open areas on hot, clear days. Even when active, adults are particularly reclusive and tend to stay hidden in tall grasses. They are more reluctant to forage in wide open loam areas, in contrast to *D. pruininus* and *D. velutinigrens*. Beetles can also be found on thick grass mats as they forage, mate or disperse. When disturbed, they use these dead grass mats as cover.

DROMOCHORUS VELUTINIGRENS JOHNSON, 1991

(FIG. 9G)

Common name

Velvet tiger beetle.

Type locality

'10 km east of Riviera, Kleberg Co, Texas'. Syntypes (3) in USNM, Washington DC.

Distribution

Dromochorus velutinigrens is currently known from south and west Texas, from the Gulf Coast south of Corpus Christi, west to Dimmit County. Few localities are known (Fig. 3), and gaps between the known occurrences may be due to a lack of sampling in the intervening areas. It is likely that *D. velutinigrens* also may be found in adjacent areas of Tamaulipas, Mexico.

In the vicinity of LaSalle and Dimmit Counties, *D. velutinigrens* and *D. chaparralensis* come in close proximity, and are sympatric in at least one site.

Diagnosis

This is a very distinctive species of *Dromochorus*. *Dromochorus velutinigrens* can be diagnosed by having strong blue, violet or green reflections throughout the entire dorsal surface, especially towards lateral margins, in conjunction with a narrow gracile body form and a pronotum with few to no setae. Males are unique among *Dromochorus* for having an all dark labrum.

The only species that could potentially be confused with *D. velutinigrens* is *D. welderensis*.

Dromochorus welderensis is black dorsally, with a faint dark-blue sheen. It has scattered to regular white decumbent setae present on the disk of the pronotum, especially along lateral margins. Males have a pale central spot on the labrum. Body form is more robust than *D. velutinigrens*.

Description

Medium- to large-sized *Dromochorus*. Body length 11.4–14.9 mm, mean ♀ 13.3 mm, mean ♂ 12.7 mm. Head slightly wider than pronotum. **Head charcoal black with velvety violet to blue sheen, with bright violet, blue or greenish reflections in supraorbital areas and anterior margin**. Fine rugosity often present on the frons and vertex. All head portions glabrous except for two supraorbital setae next to each eye. Frons concave in median area, especially in male, bulging towards slightly convex near anterior margin, clearly delimited from clypeus, gradually blending into vertex. Genae bright polished metallic blue to violet, blending to violet posteriorly, with shallow longitudinal striae gradually ending at border of vertex. Clypeus bright metallic blue and violet. Male labrum tridentate with 6–8 setae, entirely dark-brown to black with polished metallic cupreous to green reflections, rarely with a faint ochre-testaceous spot in centre; female labrum tridentate with 6–8 setae, entirely dark-brown to black with polished metallic cupreous to green reflections. **All segments of maxillary and labial palpi consistently dark-brown with metallic violet and green reflections; apical segment is not darker than other segments**. Antennae normal length, reaching back to humerus and basal third of elytron, slightly longer in male than female; scape dark testaceous to black with metallic reflections of violet, cupreous and green, with 2–3 apical setae; pedicel dark testaceous with metallic reflections of violet, cupreous and green, lacking any setae; flagellum dark testaceous, antennomeres 3–4 with metallic violet and green reflections, densely clothed in short, white setae, antennomeres 5–11 dull-textured without metallic reflections and possessing erect setae in apical rings only, covered with fine pubescence throughout.

Thorax: Pronotum 2.2–3.1 mm in length, mean ♀ 2.8 mm, mean ♂ 2.7 mm; width 2.3–3.2 mm, mean ♀ 2.9 mm, mean ♂ 2.7 mm. Pronotum charcoal black, with velvety violet to blue sheen, slightly wider than long, widest near anterior margin, width to length ratio 0.9 to 1.1, **pronotal setae absent or with few, irregular, long setae scattered throughout disk; disc smooth**, with thin but distinct median line, shallow sulci present anteriorly, and present but less well-defined posteriorly; notopleural sutures clearly defined, not visible from dorsal view; proepisternum metallic blue to violet reflections, glabrous. Elytra elongate, 6.7–9.0 mm length, mean ♀ 8.2 mm, mean ♂ 7.7 mm, shape similar in both sexes, but slightly wider in female, especially toward apical third; sutural spine absent, microserrations not present on **elytral apices; elytral dorsal surface convex, texture dull throughout, elytral coloration charcoal black with velvety violet to blue sheen, lateral margins and apex with shining blue, violet or green reflections**; elytral maculations absent; **infuscations absent; subsutural foveae absent**.

Legs: Pro-, meso- and metacoxae black with iridescent blue, violet and green reflections, with numerous setae, fewer on metacoxae; pro- and mesotrochanters with a single erect seta, metatrochanter glabrous, trochanters dark brown-testaceous; femora metallic violet to blue, with green reflections, densely clothed in decumbent white setae; tibiae brown, clothed with setae of two types: sparser brown-testaceous long setae and dense short decumbent white setae; two tibial spines present; tarsi brown-testaceous, first three dilated protarsomeres in male with dense greyish-white setal pad.

Abdomen: Venter black with metallic violet to greenish reflections throughout most surfaces. Decumbent white setae present on ventrite 1. Ventrites 2–6 have scattered short brown recumbent setae present throughout, but often abraded.

Ecology / natural history

Adults are active earlier than other species in the genus. Records are from mid-April through late June,

but year to year, emergence dates are highly variable relative to congeners and may be more dependent on spring rainfall patterns.

Our limited knowledge of habitat associations for *Dromochorus velutinigrens* is based on two populations. The type locality is a Texas A&M University–Kingsville's Site 55 Biological Research Station (Johnson, 1991) on the northern shore of Baffin Bay in Kleberg County, TX. Much of the habitat along the northern banks of the bay has been destroyed by agricultural use, primarily heavy grazing. Johnson (1991) reported that the species can be found associated with sandy road paths and grassy areas along semi-forested areas through the sites. Our team observed adults in sandy, vegetated backshore regions near the bay just below shrubby clay dunes (lomas). Individuals can be found running in and around bunches of cord grass (*Spartina spartinae*) and in relatively open sandy areas not far from the water's edge. The adults of *D. velutinigrens* are by far the most sand-tolerant of the known *Dromochorus*, as indicated by their presence also on sandy backshore areas near vegetated dunes.

Clay lomas are prevalent along the southern Texas coast from St. Charles Bay through north-eastern Mexico along the coast of Tamaulipas to Rancho Tepehauje (Tunnell *et al.*, 2002). Future surveys need to focus around clay dune formations in these regions where the beetle is currently undocumented.

Dromochorus velutinigrens also occurs more inland, as far west as Chaparral Wildlife Management Area in Dimmit and LaSalle Counties. In these areas, we believe that the species' presence may be explained by the Dilley soil series. These orange/reddish soils are classified as fine sandy loams and are darker in contrast to the type locality in Kleberg County. Extreme soil colour variability has also been observed across the ranges of *D. belfragei* and *D. pruininus*. The Dilley series extends to the North into Zavala County. Although unconfirmed, the range for *D. velutinigrens* will likely extend into this county as well.

This location is part of the greater Gulf Coastal Plain physiographic province, and may explain historical connectivity between inland and coastal populations of *D. velutinigrens*. It is probable that this inland population is not disjunct but, instead, that there has not been sufficient sampling in the intervening areas. Targeted surveys may yield other populations in sandy formations in Mexico and southern Texas.

Adults can be found among ghost crab (*Ocypode quadrata*) colonies, which *D. velutinigrens* may use for escape when pursued. The ability to hide in cracks for escape appears to be widespread throughout the genus.

DROMOCHORUS PILATEI GUÉRIN-MÉNEVILLE, 1845 (FIG. 9H)

Common name
Cajun tiger beetle.

Type locality
'Velasco, Texas' (translation). Holotype probably in MHNP, Paris (Bousquet, 2012).

Synonymy
Cicindela maga LeConte, 1875: 161. Type locality 'near Lake Ponchartrain, Louisiana'. Syntypes (2) in MCZN. Synonymy established by Sallé (1877).

Taxonomic history
This is the type species for the genus *Dromochorus*, as described by Guérin-Méneville (1845). Two remarkably green specimens were collected by LeConte and described as *Cicindela maga* (1875) from the vicinity of Lake Ponchartrain, LA.

Distribution
Dromochorus pilatei is known from south-east Texas in the vicinity of the Brazos River east to the Mississippi River in Louisiana, north to Natchitoches, LA. The Lake Ponchartrain record is uncertain. This flightless beetle has otherwise never been found east of the Mississippi River except for LeConte's specimens. There have been multiple attempts to find the beetle in this area (Graves & Pearson, 1973; D.P. Duran, pers. obs.), but these have been unsuccessful. We regard this record as a potential error until further verification.

Diagnosis
This species cannot be confused with any other *Dromochorus*. The distinctive body form, elytral coloration with bronze and green reflections, prominent green subsutural foveae and complex surface texturing are diagnostic. Some individuals have a strong green-bronze sheen over all surfaces, and this trait appears to be more prevalent towards the eastern part of the species range.

Description
Small- to medium-sized *Dromochorus*. Body length 10.5–14.7 mm, mean ♀ 13.3 mm, mean ♂ 12.4 mm. Head slightly wider than pronotum. **Head predominantly brown with cupreous to brassy reflections, green to blue to violet reflections mostly concentrated near the anterior margin and edges of the supraorbital region. In some specimens, bright green to green-blue reflections present throughout**. Fine rugosity often present on the frons and vertex. All head portions glabrous except for two supraorbital setae next to each eye. Frons concave in median area, especially in males, bulging towards slightly convex near anterior margin, clearly delimited from clypeus, gradually blending into vertex. Genae metallic blue to violet, with

shallow, longitudinal striae gradually ending at border of vertex. Clypeus bronze with green to blue reflections throughout; female clypeus more extensively green-blue to blue-violet. Male labrum tridentate with 6–8 setae, entirely pale ochre-testaceous, with a thin dark-brown to black border; female labrum tridentate with 6–8 setae, entirely dark-brown to black with polished metallic cupreous to green reflections. **Maxillary palpi pale yellow-ochre; apical segment dark-brown to black, often with metallic purple and green reflections**. Labial palpi coloured similarly to maxillary palpi. Antennae normal length, reaching back to humerus and basal third of elytron, slightly longer in male than female; scape dark testaceous to black with metallic reflections of violet, cupreous and green, with 2–3 apical setae; pedicel dark testaceous with metallic reflections of violet, cupreous and green, lacking any setae; flagellum dark testaceous, antennomeres 3–4 with metallic violet and green reflections, densely clothed in short, white setae, antennomeres 5–11 dull-textured without metallic reflections and possessing erect setae in apical rings only, covered with fine pubescence throughout.

Thorax: Pronotum 1.8–3.2 mm in length, mean ♀ 2.8 mm, mean ♂ 2.6 mm; width 2.3–3.4 mm, mean ♀ 2.9 mm, mean ♂ 2.7 mm. Pronotum brown with cupreous, brassy or violet reflections; some specimens with green to green-blue reflections throughout, slightly wider than long, widest near anterior margin, width to length ratio 1.0 to 1.2, setae sparse to regularly spaced, mostly present along lateral third of dorsal surface; disc finely rugose, with thin but distinct median line, with well-defined shallow sulci present anteriorly and posteriorly; notopleural sutures clearly defined, not visible from dorsal view; proepisternum black with weak to strong iridescent violet reflections, glabrous. Elytra elongate, dorsal surface convex, 6.4–9.0 mm length, mean ♀ 8.2 mm, mean ♂ 7.6 mm, shape similar in both sexes, but slightly wider in female, especially toward apical third; sutural spine absent, microserrations not present on elytral apices; elytral **surface dull with complex texturing and infuscations, with regular small pits present throughout disk, as well as larger foveae. Bright green or blue reflections generally present in most to all pits and foveae**.

Legs: Pro-, meso- and metacoxae dark-brown to black, may have iridescent blue reflections, scattered setae on pro- and mesocoxae, fewer on metacoxae; pro- and mesotrochanters with a single erect seta, metatrochanter glabrous, trochanters dark brown-testaceous; femora dark-brown black with metallic violet and bronze reflections, densely clothed in decumbent white setae; tibiae testaceous brown, clothed with setae of two types: sparser brown-testaceous long setae and dense short decumbent white setae; two tibial spines present; tarsi brown-testaceous, first three dilated protarsomeres in male with dense greyish-white setal pad.

Abdomen: Venter mostly dark-brown to black with faint violet reflections. Erect brown setae present on ventrite 1. Ventrites 2–6 have sparse short, brown, erect setae present throughout, but often abraded.

Ecology / natural history

Adults appear to be active from mid-May to mid-July. *Dromochorus pilatei* can be found in significant numbers during peak adult activity (early to mid-June), along shaded, dark soil trails in riparian zones or near the banks of bayous, lakes and salt marshes. Of the *Dromochorus*, *pilatei* has the strongest affinity for heavily forested areas. The species is tightly associated with blackish, rich soils with high humus content, which are produced in the forest via decaying vegetation. In contrast, *D. pruininus* and *D. belfragei* are associated with disturbed clay deposits that contain less organic matter (red iron oxidized clays or black clay loam). In our observations, *D. pilatei* apparently avoids the lighter coloured soils that can also be present in its habitat.

This species can be found foraging/mating along man-made trails, disturbances or semi-open vegetated areas of applicable forest. Beetles appear to be concentrated on the edges of trails, sometimes with moderate to thick vegetation. *Dromochorus pilatei* is the only member of the genus that has been collected at lights at night (J. Back, pers. comm.). However, it was collected in small numbers, and it is likely that its presence was due to a high density of prey in the area, created by the lights. Traditionally thought to be crepuscular and perhaps nocturnal (Graves & Pearson, 1973) in this habit, we now know this species is active throughout the day in well-shaded areas.

DISCUSSION

To explore the biodiversity of this poorly studied group of tiger beetles, we employed a 'taxonomic congruence' approach, where multiple datasets were separately analysed and species hypotheses were evaluated based on the consensus of all datasets. First, we generated species hypotheses based on patterns of reciprocal monophyly across the mitochondrial and nuclear gene datasets, and we tested these hypotheses based on their congruence with population structure, conventional morphological measures, ecological divergence, and geographic isolation. We found broad consensus among these datasets (Table 4), supporting the existence of eight species within the genus *Dromochorus*. This more than doubles the number from the last North American catalogue (Freitag, 1999), which recognized only three. Except in one area of geographic contact (discussed

Table 4. Congruence between datasets with respect to the eight putative species of *Dromochorus*. Each X indicates that a hypothesized species could be circumscribed from other such species based on that dataset

Species	mtDNA	Distance	Pop. Structure	Ecology	Morphology
Dromochorus pilatei	X	X	X*	X	X
D. velutinigrens	X	X	X	X	X
D. welderensis	X	X	X	X	*Indistinct from D. chap*
D. chaparralensis	X	X	X	X	*Indistinct from D. weld*
D. minimus	X	X	X	X	X
D. pruininus	X	X	X	X	X
D. belfragei	X**	X**	X**	X	X
D. knisleyi	X**	X**	X**	X	X

mtDNA: forms monophyletic clade.
Distance: forms monophyletic clade.
Pop. Structure: forms individual group in optimum pop # estimate.
Ecology: forms distinct clusters at Euclidean and Manhattan distances.
Morphology: statistically distinct via nested ANOVA.
**Dromochorus pilatei* was not directly assessed in targeted comparisons, as the species monophyly was unambiguous based on the mtDNA genealogy, multilocus genotyping tree, ecological divergence, as well as multiple diagnostic morphological characters.
**Monophyletic except for populations from Bexar County, TX.

below), all eight species were found to be monophyletic with respect to the mtDNA genealogy, and overwhelmingly congruent in comparison with subsequent datasets.

Avoiding under- or overestimating species diversity

Morphological characters have been used more than any other type of data when describing/circumscribing eukaryote species. Morphology alone may over-split polymorphic taxa, and is well-known to 'lump' species together, especially for recently speciated groups. Conversely, mtDNA markers have an elevated rate of evolutionary change relative to most nuclear markers (Zhang & Hewitt, 1996), and taxonomy that is based exclusively on mtDNA may tend to overestimate the true diversity (Rubinoff, Cameron & Will, 2006; Song *et al.*, 2008). This tendency to over-split is exacerbated in cases where species have poor vagility (Bond & Stockman, 2008). Within the larger insect taxonomy community there has been reluctance to incorporate other non-traditional taxonomic characters such as phenology, ecology and behaviour into alpha taxonomy. Interestingly, these non-morphological, non-genetic characters are regularly used to differentiate species of birds (e.g. *Empidonax* flycatchers), and there is no reason to believe that similar characters would be any less informative in insects. The recognition of cryptic species may greatly increase the known biodiversity; the authors of a recent study estimate that there may be double the number of presently accepted bird species, when cryptic species are factored (Barrowclough *et al.*, 2016)

Even though tiger beetles are one of the most popular and taxonomically well-studied groups of insects (Knisley & Schultz, 1997), our multi-dataset congruence analysis increases the number of species in this genus, from three or four to eight. Prior North American tiger beetle taxonomists have generally accepted either three species (Freitag, 1999) or four species (e.g. Johnson, 1991; Bousquet, 2012), depending on whether *D. pruininus* was recognized as a valid taxon. Part of the reason for underestimating *Dromochorus* species diversity is the historical reliance on morphological characters exclusively. We recognized that there was a paucity of traditional morphological characters in *Dromochorus* (e.g. reduced number of setae in most areas, lack of maculations) and approached the taxonomic problem by first conducting a thorough congeneric phylogeographic approach (Funk & Omland, 2003), sampling from as many geographic areas as possible, for all distinct populations and putative species within the genus. This allowed for the generation of a well-resolved mtDNA genealogy that contained multiple statistically supported and well-separated clades, and these were treated as putative species hypotheses to be tested. It is well-known that mtDNA genealogies may identify more monophyletic groups than would phylogenies based on multiple genetic markers (Hudson & Coyne, 2002). Therefore, the number of species could be overestimated, if mtDNA markers are used exclusively. However, inspection of the multilocus genotyping data allowed us to arrive at the same conclusion at the mtDNA genealogy, bolstering the strength of our initial inferences. Each of these clades represented geographically constrained sets of populations, and we followed up with comparative morphological assessment. During this process, it was observed

that the coloration of the maxillary and labial palps co-varied with the structure observed in the mtDNA tree. Consequently, we could identify multiple new morphological characters (palp coloration, and contrast between apical and subapical segments) that had never before been utilized in tiger beetle taxonomy. In the process, other informative characters were found, such as the absence or extreme reduction of pronotal setae on *D. velutinigrens*, an already described species. In contrast, *D. chaparralensis* and *D. welderensis*, were indistinguishable based on fixed morphological synapomorphies or morphometrics (Table 4). Interestingly, these two cryptic species were more ecologically differentiated than other pairwise species comparisons in the '*velutinigrens* group' (Table 3), based on Euclidian distance values from the ecoregion data. *Dromochorus chaparralensis* was more differentiated from *D. welderensis* (132.4) than from other geographically proximate species within that group, such as *D. velutinigrens* (77.2) or *D. minimus* (69.5), which are distinguishable based on diagnostic morphology (see dichotomous key) or morphometrics (Fig. 6), respectively.

Our approach could be applied to uncover other cryptic species, even in the relatively well-studied tiger beetles. Many species of tiger beetles in the subtribe Cicindelina contain multiple geographically disjunct populations (e.g. *Cicindela willistoni*, *Ellipsoptera nevadica* and *E. puritana*), some of which may exhibit differences in phenology or ecology. Often, these sets of populations are referred to as separate subspecies, provided there is any variation in color, maculation size (i.e. width of white markings) or average body size to accompany the geographic isolation. The subspecies concept is fraught with problems, as laid out in Wilson and Brown (1953) and more recently discussed in Mallet (2001). Current attempts to rigorously test the validity of morphologically defined subspecies have found that few are supported as evolutionarily meaningful entities (e.g. Zink, 2004). However, some of these taxonomic subspecies may turn out to represent fully separate species, if evaluated using a congruence method, such as ours. Moreover, phenology, ecology and behaviour are currently underutilized for species inference in tiger beetles (but see: Vick & Roman, 1985; Duran & Roman 2014).

Potential hybrid zones

Our study underscores the importance of using this congruence method, not only for species discovery, but to further elucidate evolutionary history and assess ongoing processes, such as gene flow. The initial mtDNA tree yielded largely allopatric and monophyletic clades; however, in one area of sympatry between populations of putative species, there was substantial polyphyly observed between *D. belfragei* and the cryptic species *D. knisleyi*, both of which occur in Bexar County, TX. Despite clear morphological and ecological differentiation between those otherwise distinct entities, some individuals appeared to have introgressed mtDNA. This was further observed in the multilocus genotyping dataset. Both the population-level tree and the STRUCTURE plots show that almost all the incongruence is occurring based on a single *D. belfragei* population that comes within a few kilometers of the Balcones Escarpment in south central Texas. In this area, these species come into geographic contact, and habitats for each species overlap. *Dromochorus knisleyi* is found on the north-western side of the escarpment, in the region known as the 'Hill Country', and has only been observed in mature juniper woodlands. *Dromochorus belfragei* occurs in semi-open grassy areas with cracked loam, habitat, which mostly occurs east and south of the escarpment. During 2014, the first author visited a natural area only 2 km from the introgressed *D. belfragei* population, where phenotypically pure examples of *D. knisleyi* and *D. belfragei* were observed in their respective typical habitats. On trails that cut through both of these habitats, pure individuals of both species were found, as well as individuals that appeared to have a mix of both parental species' characteristics. As observed in many other taxonomic groups, otherwise 'good' species may have geographically restricted contact zones where hybridization may occur. Despite many of the eight *Dromochorus* species ranges being in close geographic proximity to others (Fig. 3), we only discovered one other location where multiple species occurred (*D. velutinigrens* and *D. chaparralensis*, Chaparral WMA).

A surprising result was the discovery that *D. minimus* was recovered in different parts of the topology in the mtDNA genealogy and the population-level tree generated with the multilocus nuclear data. It is possible that *minimus* is derived from the '*velutinigrens* group' historically, but more recent hybridization and introgression is responsible for its placement on the mtDNA tree. Despite the large phylogenetic divergence between the two major mtDNA clades, this result suggests species may be interfertile when they come in contact. Although *D. minimus* individuals do form a monophyletic clade, they are contained within the larger *D. knisleyi* and Bexar County *D. belfragei* clade. Their placement might be best explained by recent contact with nearby *D. knisleyi* and replacement of their mtDNA haplogroup. Geographically and ecologically, *D. minimus* is more similar to the '*velutinigrens* group' as well, and the multilocus genotyping data places them in that group.

CONCLUSION

Our congruence approach allowed for the discovery of four new *Dromochorus* species (*D. knisleyi*, *D. welderensis*, *D. minimus* and *D. chaparralensis*), the validation of one previously ambiguous taxon (*D. pruininus*), and

a doubling of the diversity of the genus. By identifying putative cryptic species via the mtDNA genealogy and multilocus genotype trees, we were able to test these for congruence with morphological and ecological datasets. This procedure yielded new informative morphological characters that had not previously been used to resolve taxonomic relationships within the genus, and these may be informative in other genera within the larger tribe. Moreover, this information allowed for reassessment of the morphological characters of previously described species, and new diagnostic synapomorphies were identified. We provided morphological descriptions of new species, re-descriptions of previously named species (*D. pilatei* Guérin-Méneville, *D. belfragei* Sallé and *D. velutinigrens* Johnson), and created an updated dichotomous key to the genus. The present study also yielded new and updated natural history/ecological characteristics for the genus, as well as individual species. Lastly, we hope the workflow of our integrative approach can be used directly for other taxa, or inspire improved methods to identify cryptic biodiversity in both poorly studied and well-known groups.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Table S1. Locality data for specimens examined in this study. Museum acronyms are defined in Material and Methods. Ecoregion names correspond to the EPA Level III ecoregions indicated in Table 1. DNA numbers correspond to the three digit identifiers used for each specimen in the mtDNA genealogy illustrated in Figure 2.